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THE PATHOLOGY OF HIGH-ALTITUDE FROSTBITE *

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This study of the morbid anatomy of frostbite incurred by airmen exposed to severe cold at high altitudes is the second in a series of reports dealing with the reactions of tissues to injury by cold. It is based on material received at the Army Institute of Pathology from military sources.†

Frostbite of aviators was noted during the First World War [1,2] and the Spanish Civil War,[3] but until bombing from high altitudes had become a regular practice in World War II it was not a common condition. Among men of the Eighth Air Force high-altitude frostbite was at one time the second most frequent battle injury.[4] The experience of the Army Air Force with this problem has been recorded by Davis, Scarff, Rogers, and Dickinson [5] and that of the Royal Air Force by Cade.[6]

CLINICAL DATA

The 20 patients from whom material was obtained for this study (Table I) incurred frostbite during raids in B-17 and B-24 bombers at altitudes of 20,000 to 27,500 feet and at temperatures of $-30°$ to $-50°$ C. The men were in their late teens or early twenties and with one exception were white Americans. The majority were tail gunners, but waist and turret gunners are included in the group. Five had incurred frostbite during previous missions.

The briefest exposure was 1 minute and the longest 5 hours. The usual reason for exposure was removal of electrically heated gloves to deal with a frozen oxygen mask or tubing, but burned-out wiring and

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† Most of the specimens were obtained from patients studied at the 2nd General Hospital by one of the authors (R. A. K.), but material was also contributed by Lt. Col. Robert Hebbel, Lt. Col. Harold L. Stewart, and Capt. Robert H. Maschmeyer, from the Letterman and 26th General Hospitals and the 49th Station Hospital. The photographs of the lesions were selected from the collection of Major John E. Scarff; the photomicrographs were taken by Mr. Roy Reeve.

damage to equipment by flak were sometimes responsible. Six men had anoxia at the time of exposure; the reactions to lack of oxygen ranged in severity from partial blackout to prolonged coma. The anoxia is of interest because inadequate oxygenation has been thought to augment injury by cold.[5]

The fingers, affected more often than other parts of the body, were involved in this order of frequency: fourth, index, third, second, and thumb. One man had frostbite of the toes and 4 had damage to the face in addition to injury of the fingers. Sheeley [7] reported that frostbite of the face of aviators remained a problem after the incidence of frostbite of the fingers had been reduced. One patient had frostbite of the buttock (Fig. 1), as a consequence of urinating into his flying suit.

During exposure the fingers became numb, stiff, shrunken, and hard; they were described as yellow-white and waxy. As the frostbitten fingers thawed, in the navigator's compartment or radio room, during descent of the plane or after landing, pain and swelling set in. Immersion of the fingers in cold water deferred the edematous reaction, but after the hands were removed from the water cyanotic discoloration or redness, edema, and blisters (Fig. 2) appeared. Bullae sometimes developed a few hours after exposure, especially if the hands had been excessively warmed; they were almost invariably present within 24 hours.

Damage of the mildest type resulted only in shedding of the skin and nails; there was no significant loss of tissue. Severe injury was followed by gangrene of the digits (Figs. 3 and 4), sometimes as soon as 8 days after exposure, although usually 2 to 3 weeks were required for its full development.

Microscopic Changes

Most of the material consisted of amputated gangrenous fingers, which showed only late lesions. In a few instances tissue was taken for biopsy prior to amputation.

Early Lesions

The description of the early lesions is based primarily on the study of a specimen obtained for biopsy from a finger 3 days after exposure. The finger subsequently became gangrenous.

The specimen included the roof of a vesicle and a portion of the subcutaneous tissue. The space formed by cleavage at the dermo-epidermal junction contained precipitated protein, leukocytes, and red cells. The outer epithelial layers were necrotic, while in many places the inner portion of the malpighian layer and the basal layer were

TABLE I

Data from Twenty Cases of High-Altitude Frostbite

Case number	Age of patient	Patient's position in plane	Altitude, thousands of feet	Temperature	Circumstances of exposure	Duration of exposure	Complications	Number of days to		Portion of body involved
								Gangrene	Amputation	
1	22	Tail gunner	26	−30°C.	Oxygen mask froze Ungloved hand	1 hour	Anoxia	15	47	Fingers
2		Tail gunner	27	−45°C.	Flak cut wiring of heated suit	Few minutes	Anoxia; abdominal wound	12		Fingers
3							Head wound			Fingers
4	23	Tail gunner			Oxygen mask froze Insufficiently heated gloves		Anoxia	15	42	Fingers
5			26.5	−45°C.	Gloves failed to heat			27		Fingers
6	19	Tail gunner	24		Oxygen mask froze Insufficiently heated gloves		Anoxia			Fingers
7	22	Tail gunner	27	−48°C.	Gloves failed to heat	5 hours	Previous frostbite		42	Fingers, toes, cheeks
8	24	Turret gunner			Disconnected suit Hydraulic fluid froze on gloves			8	42	Fingers, cheeks
9	22				Oxygen mask froze Insufficiently heated gloves		Previous frostbite		56	Fingers
10	23				Suit burned out No gloves	4 hours	Previous frostbite	8	55	Fingers
11					Oxygen mask froze Insufficiently heated gloves				59	Fingers
12		Tail gunner	26.5	−45°C.	Gloves failed to heat		Previous frostbite	27	71	Fingers
13	20	Tail gunner			Oxygen mask froze Removed gloves	1 hour		27	71	Fingers
14	22		20	−40°C.					109	Fingers
15									1 (year)	Fingers
16		Tail gunner	27.5	−48°C.	Oxygen mask froze Wiring burned out Gloves failed to heat	1 hour	Anoxia			Fingers, cheeks
17		Turret gunner	25	−32°C.	Urinated in suit		Previous frostbite			Buttock
18		Waist gunner	26	−40°C.	No heated clothes					Fingers
19		Waist gunner			Oxygen mask froze Removed gloves	1 minute	Anoxia			Fingers
20										Fingers, face

preserved. The largest groups of viable cells were at the apices of the rete pegs in the stripped-off epidemis (Fig. 5). Fibrinoid material lay amidst the loosened epithelium. Some cells were vacuolated or dyskeratotic and had enlarged bizarre nuclei; multinucleated syncytial giant cells were prominent (Fig. 6). There was precipitated material in the lumina of the sweat glands, and chromatin débris was present in the degenerated lining epithelial layer (Fig. 7). Even in better preserved glands the cells were swollen.

Necrotizing changes were prominent in the subcutaneous vessels, especially those in the fat lobules. The arterioles and venules had homogenized, hyalinized, smudgy walls, in which erythrocytes and bits of chromatin were embedded (Fig. 8). The nuclei of the degenerated endothelial cells were pyknotic or fragmented. Packed masses of erythrocytes and hyaline material plugged the small vessels; the red cells appeared conglutinated. The damaged vessels were not necessarily those most tightly crowded with erythrocytes. A scattering of leukocytes infiltrated the adipose tissue (Fig. 7), but the panniculitis was not accompanied by inflammation of the overlying dermis or of the adjacent fibrous septa.

Late Lesions

The description of the late lesions is a composite of observations made on material obtained for biopsy or at operation 2 to 50 weeks after exposure. Except for the vascular alterations, the histologic changes varied little from specimen to specimen.

With severe injury gangrene of the peripheral portion of the finger involved the deep structures as well as the superficial tissues. Necrosis was restricted to the epidermal roofs of vesicles when the patient had incurred only mild damage.

In mummified tissues the collagen appeared desiccated; the vessels were filled with homogenized masses of hemolyzed red cells. Bacteria had invaded the necrotic tissues, and colonies of organisms were particularly evident along the course of the hair follicles. There was extensive cellulitis in regions adjacent to areas of gangrene.

Both hyperplasia and atrophy of the epidermis were evident at the line of demarcation between gangrenous and viable portions of the finger and at the edges of ulcerated regions. In places a regenerated thin epithelial layer without pegs covered previously denuded areas, and pustular crusts often overlaid a subepithelial zone of inflamed granulation tissue (Fig. 9).

The sweat glands showed a variety of degenerative changes. Vacuolation of the epithelial cells and dilatation of the coils gave a microcystic appearance to some glands. Inspissated secretion filled a few

ducts, and the linings of others showed squamous metaplasia. Thickened basement membranes surrounded atrophic coils, and the fibrofatty lobules about the sweat glands showed depletion of fat and mucohyaline degeneration of the connective tissue. There were occasional cystic epidermal inclusions which may have arisen from sweat ducts. Inspissated desquamated material in some of them had provoked a granulomatous reaction in which foreign body giant cells were prominent.

There was diffuse sclerosis, particularly in older lesions; in some instances the subepithelial fibrosis resembled that of a keloid (Fig. 10). Usually there was a moderate infiltration by a variety of cells, but in one case a peculiarly even distribution of large mononuclear elements throughout the tissues was encountered (Fig. 16). Fibrosis and hyalinization often involved nerves, and there was perineural sclerosis (Fig. 11). Panniculitis, necrosis of fat, and lipoid phagocytosis were observed. Mucinous connective tissue replaced some adipose lobules, and in others the fat was atrophic and scarred (Fig. 12). Here and there scattered fat cells or a focal lipogranulomatous reaction marked the site of destroyed adipose tissue.

The evolution of the lesions was most clearly reflected in the progressive vascular changes; thrombosis, organization of thrombi, and obliterative angiitis were all encountered. The thrombi, which were of the agglutinative erthrocytic variety, contained little, if any, fibrin. They persisted in large vessels for as long as 6 weeks after exposure (Fig. 13), but only organized and recanalized plugs were encountered in fingers amputated after that interval (Fig. 14). Even in the organized thrombi clumps of well preserved erythrocytes and hemosiderophages were evident. The single or multiple new channels in the recanalized vessels sometimes had well developed musculo-elastic coats (Fig. 15). Some nonthrombosed arteries exhibited loose mucinous or lamellated fibrous thickening of the intima. There were also proliferative endophlebitic changes. The vessels coursing through relatively normal tissue, as much as 2 cm. above the zone of gangrene, were sclerotic. Scattered macrophages laden with hemosiderin marked the sites of old hemorrhages; they were often concentrated about vessels and nerves. Occasional vessels had mural hemorrhages. Chronic lesions exhibited many thin-walled collateral vascular channels (Fig. 16).

Comment

The lesions of high-altitude frostbite, like those of trench foot, are characterized by the prominence of thrombosis and vascular occlusion. The peripheral gangrene must be attributed to ischemia rather than to direct freezing of the tissues. The necrotizing vascular changes in the

earliest lesion bore no unequivocal relation to thrombotic plugs; they resembled the changes of perniosis and may have represented a direct effect of cold.

Although the thrombi are clearly agglutinative, there is no satisfactory explanation for the conglutination of red cells. In a study of experimentally produced frostbite, Lange [8] observed *in vivo* clumping of erythrocytes which could not be accounted for by loss of fluid from the vessels. In an earlier report he and his co-workers [9] pointed out that the clumps of red cells in the vessels could be washed free during the first 72 hours after exposure to cold but then formed cohesive thrombi. They discovered that heparinization prevented both thrombosis and gangrene.

Involvement of the adipose tissue was less in high-altitude frostbite than in trench foot, but the suggestion, previously advanced,[10] that cold has a special action on tissues rich in lipoid has received additional support from other sources. Two cases of pulmonary fat embolism following exposure to cold have been studied,[11, 12] and an example of crystallization of fat in an early lesion of trench foot has been encountered since the report on trench foot was published.

The material was not suitable for study of the sympathetic nerves. In the lesions of trench foot there was relative sparing of the vasoconstrictor fibers in mixed nerve trunks,[10] an observation at variance with that made by Blackwood in a study of immersion foot.[13] He noted damage to all fibers, especially the smaller and unmyelinated ones, although his observations, unfortunately, were erroneously reported in the paper on trench foot.[10] Blackwood and Russell [14] described degeneration of nerve and muscle, in the absence of vascular changes, following experimental exposure to cold.

High-altitude frostbite, a variety of "true frostbite," [5a, 15] is a classic example of the injury produced by "freezing," and trench foot exemplifies that caused by "chilling." The fact that no significant differences were noted between the lesions of high-altitude frostbite and those of trench foot [10] supports the concept that the reactions of tissues to cold exhibit a fundamental unity.[16] The differentiation between "chilling" and "freezing" injuries is becoming less sharp. For example, Lake [17] stated that even in cases of true frostbite the major part of the reaction may be of the secondary neurovascular type which follows chilling. Even Ungley, Channell, and Richards,[18] who wrote, "There has been a tendency to confuse the condition [immersion foot] with frostbite. This confusion persists even at the present day," admit, in the same paper, that "Cases of this nature are exceptional, but they suggest that the distinction between frostbite and immersion foot may not be so clear-cut as has been believed heretofore."

The superficial dissimilarities between the changes induced by freezing and those resulting from chilling reflect no fundamental difference in pathogenesis. The extreme degree of cold which causes frostbite, even if it acts for only a brief period, results in sharply demarcated and uniform damage. Prolonged exposure to a lesser degree of cold permits secondary factors, such as differences in sensitivity of tissues and local metabolic and vascular conditions, to exert their effects; as a consequence the reaction is unevenly distributed and irregularly demarcated. The results of varying exposures to different degrees of cold are roughly comparable to those produced by varied exposures to diverse amounts of radiant energy, another injurious physical agent. A necrotizing dose of irradiation will destroy all tissue within the field, while a smaller dose is followed by a complex of irregularly distributed destructive and reactive lesions, in the development of which secondary factors play important rôles.[19]

Summary

Study of the frostbite incurred by aviators during bombing missions at high altitudes disclosed that the fingers were the most commonly damaged structures, although injury to the toes, face, and buttock was also encountered. The most prominent features of the morphologic changes were agglutinative thrombosis and vascular lesions. Gangrene probably resulted from ischemia, not from freezing. The similarity of the lesions to those of trench foot supports the view that the reactions of tissues following various types of exposure to cold exhibit a fundamental unity.

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DESCRIPTION OF PLATES

Plate 29

Fig. 1. Case 17. Frostbite of buttock.

Fig. 2. Case 2. Frostbite of fingers. Bullae, 2 days after exposure.

Fig. 3. Case 4. Frostbite of fingers. Gangrene, 15 days after exposure.

Fig. 4. Case 1. Frostbite of fingers. Gangrene, 15 days after exposure.

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Friedman and Kritzler   High-Altitude Frostbite

PLATE 30

Early lesions, 3 days after exposure; biopsy specimen (case 1).

FIG. 5. Epidermal roof of vesicle. Preservation of epithelial cells at tips of pegs. × 105.

FIG. 6. Epidermal roof of vesicle. Bizarre and multinucleated epithelial elements. × 400.

FIG. 7. Degeneration of sweat glands. Leukocytic infiltration of adipose tissue. × 230.

FIG. 8. Necrosis of vascular walls. × 400.

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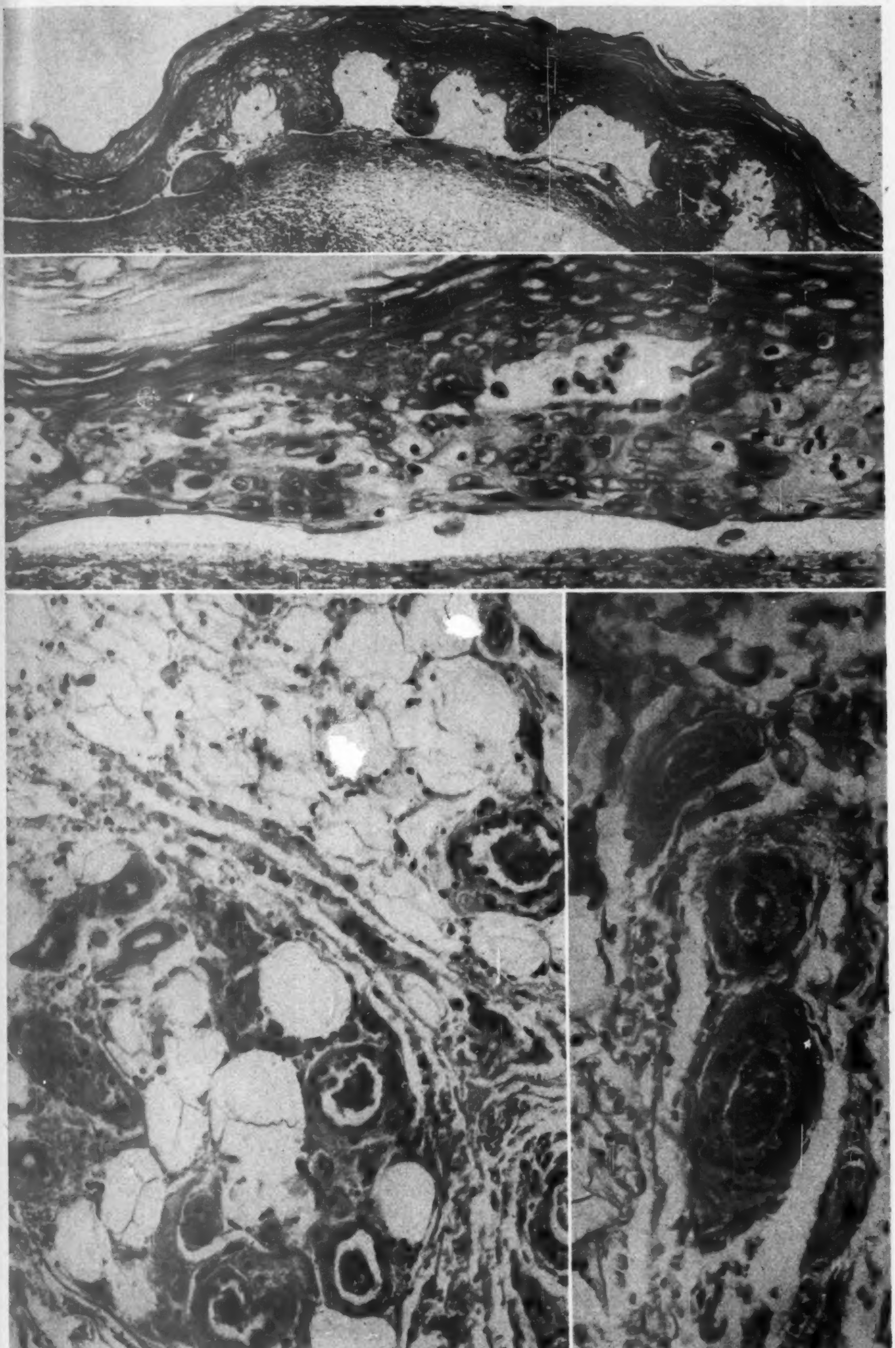

Friedman and Kritzler High-Altitude Frostbite

PLATE 31

FIG. 9. Case 13. Thin regenerated epidermis covering inflamed granulation tissue, 10 weeks after exposure. × 130.

FIG. 10. Case 15. Subepidermal fibrosis, 1 year after exposure. × 75.

FIG. 11. Case 13. Perineural sclerosis and inflammation, 10 weeks after exposure × 70.

FIG. 12. Case 15. Fibrosis of fat, 1 year after exposure. × 145.

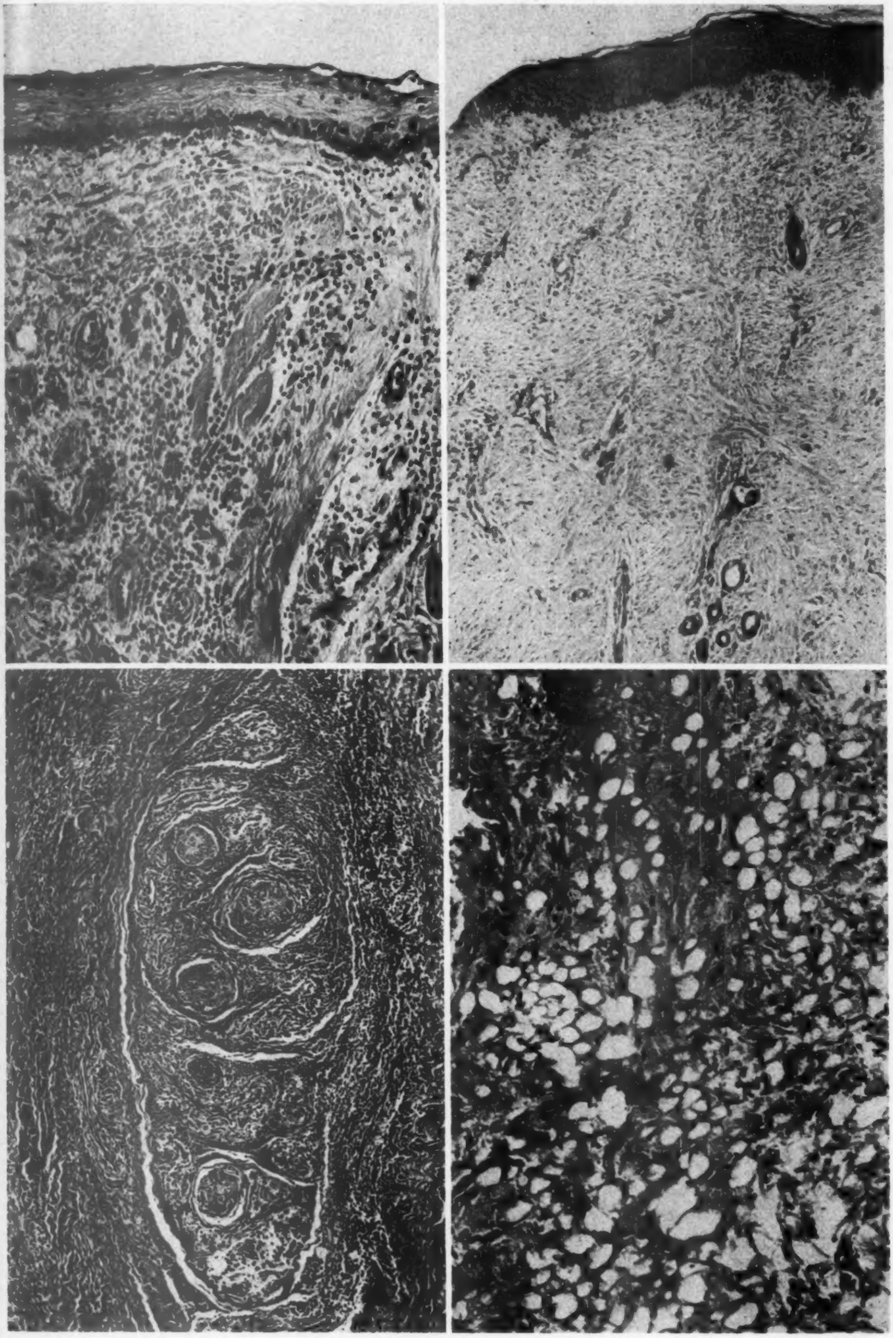

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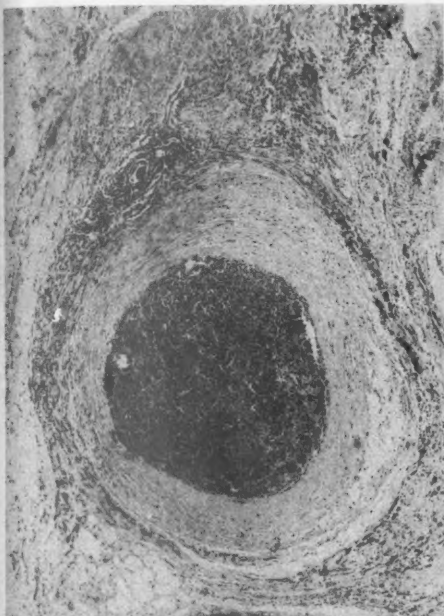
PLATE 32

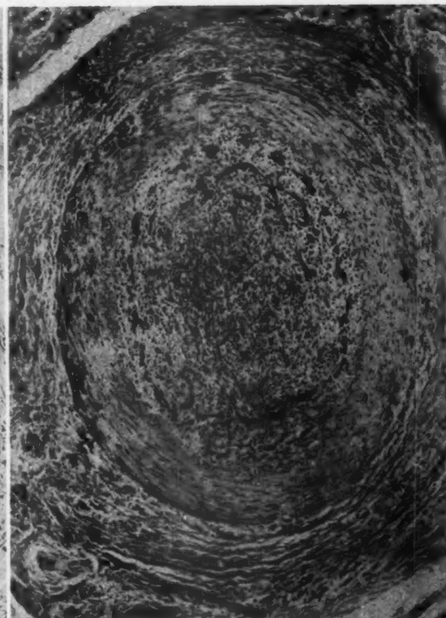
FIG. 13. Case 8. Agglutinative erythrocytic thrombosis, 6 weeks after exposure. × 60.

FIG. 14. Case 13. Organized thrombus, 10 weeks after exposure. × 40.

FIG. 15. Case 13. Recanalized vessel, 10 weeks after exposure. × 140.

FIG. 16. Case 14. Ectasia of collateral vessels and infiltration of large mononuclear elements in late lesion, 109 days after exposure. × 50.





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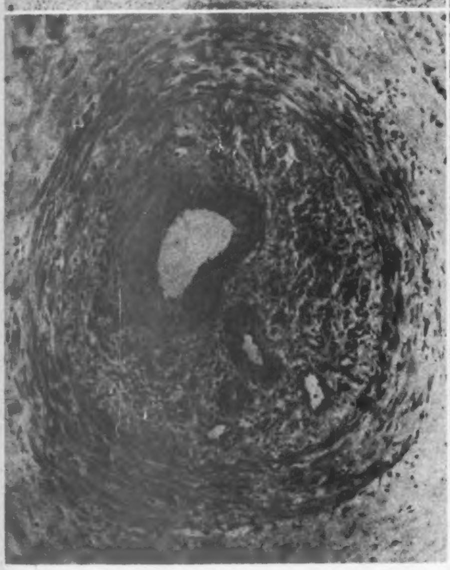

16

Friedman and Kritzler High-Altitude Frostbite

TUBEROUS SCLEROSIS WITH CONGENITAL TUMORS OF HEART AND KIDNEY

Report of a Case in a Premature Infant *

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The complex of tuberous sclerosis is of particular interest to pathologists and embryologists as it provides a striking example of the possible relationship between faulty cellular development and the process of neoplasia. The purpose of this paper is to describe the occurrence of tuberous sclerosis and other developmental defects in a premature infant of approximately 8 months' gestation who died 3 days after birth. This anomaly was associated with multiple rhabdomyomatous nodules of the heart and adenomatous foci in the kidneys. In addition, one of the nodules in the brain had assumed a distinctly neoplastic character. The cardiac neoplasms were of the type described by Batchelor and Maun [1] as "congenital nodular glycogenic tumors" and this instance is the 64th of this condition to be reported. Although tuberous sclerosis with or without malformations or tumors in other organs has been described in infants and young children by Globus and Selinsky,[2] Stewart and Bauer,[3] Farber,[4] Yater,[5] Hueper,[6] Labate,[7] Hillman,[8] Stewart,[9] and others, in so far as I can determine, this is the youngest infant in whom these changes have been observed.

Report of Case

A Negro girl, weighing 3 lbs. 15½ oz. and measuring 18 inches in length, was born on February 14, 1944. The mother, 19 years of age, had had swelling of hands and feet and headache during the last months of pregnancy. In the hospital the diagnosis of toxemia of pregnancy was made in view of a blood pressure of 165/110 mm. Hg, 2 plus edema of the lower extremities, and 4 plus albumin in the urine. It was her first pregnancy. Wassermann and Kline tests of the mother's blood were negative. The delivery was normal. The baby was thought to be in fair condition at birth and breathed spontaneously. Slight cyanosis was noted subsequently, but her condition did not become alarming until February 17th, when the cyanosis became marked and breathing difficult and gasping. The temperature rose to 100.5° F. and the baby died during the afternoon of the third day.

Gross Examination

The body was that of a small, poorly developed Negro baby girl weighing 1655 gm. No abnormalities of the skin or nails were noted.

The heart lay free within the pericardial sac and showed a smooth, round, button-like tumor projecting from the apex (Fig. 2). This mass measured 2.5 cm. across and 2.2 cm. in its superior-inferior

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diameter. When viewed from the side the nodule was somewhat triangular, measuring 1.4 cm. in thickness where its base was attached to the heart, and only 0.3 cm. at its apex. Two smaller nodules were present about the base of the main mass and a separate nodule, 2 mm. in diameter, projected from the lateral aspect of the right ventricle. Within the right atrium a subendocardial nodule, 1 mm. in diameter, was located just above the attachment of the tricuspid valve. On section these nodules were composed of reddish tan tissue of nearly the same appearance as the myocardium but of lighter color. They were well delineated.

The lungs were congested and contained irregularly distributed, firm, subcrepitant areas.

Stripping of the renal capsules revealed prominent fetal lobulations. Visible on their surfaces were reddish gray areas, measuring from 1 to 4 mm. in diameter. They did not project above the surface but were imbedded in the periphery of the cortices.

The outstanding abnormality of the brain to be noted on external examination was a hard nodule in the tip of the right frontal lobe. The convolutions over the nodule were distorted, broad, and blunt. The mass measured 3 by 1.5 by 3 cm. and on section consisted of firm reddish gray tissue that was well demarcated from the adjacent brain substance (Fig. 1). The cerebral convolutions presented no other definite abnormality on inspection, but on palpation the surfaces of the cerebral hemispheres revealed nodular areas that were firmer than the adjacent soft cerebral tissue. A glistening gray nodular mass, measuring 1.2 cm. in greatest diameter, projected into the anterior horn of the lateral ventricle just medial to the left caudate nucleus (Fig. 2). A slight nodular elevation of the cerebral tissue was also visible in a similar position on the opposite side. Scattered through the brain, but particularly in the regions of the basal ganglia, were firm, nodular, grayish white areas. One well delineated nodule, measuring 0.5 cm. in diameter, was found close to the surface of the right occipital lobe. The other areas which imparted a firm nodular quality to the palpating finger showed no macroscopic abnormality.

Microscopic Examination

Heart. The myocardial tumors presented a markedly vacuolated appearance, identical with that of many of those previously described in the literature. The huge cells were usually rounded or ovoid, but often had crinkled cell boundaries. The cytoplasm of some was completely clear, but more often was finely granular or reticulated, and many of the clear cells had a thin rim of pale-staining cytoplasm. The nuclei, when present, varied between central and peripheral positions.

No mitotic figures were found. Striated myofibrils were situated between and along the borders of the large cells and often radiated from a central nucleus, so as to form the so-called spider cells (Fig. 3). The cross striations were accentuated with the aid of trichrome staining methods. The larger tumor nodules were sharply demarcated from the adjacent myocardium, but some of the smaller ones blended imperceptibly with the normal myocardial fibers. As many as eight nodules, only three of which were visible to the naked eye, were found in one section, measuring 1 by 0.5 cm. Nodules varied in size from the large tumor at the apex of the heart to tiny foci consisting of only three or four large vacuolated or granular cells. Best's carmine stain showed red glycogen granules in the cytoplasm of some of the cells, even after preservation of the heart in formalin for 18 months.

Kidney. In the peripheral portions of the renal cortex were large, dilated, tubular structures, usually arranged in groups of from three to twenty, but occasionally singly. These were lined by large cuboidal and columnar epithelial cells, sometimes in a single layer, but frequently several layers in thickness and often piled up and projecting into the lumina in papillary fashion (Fig. 4). Their cytoplasm was generally granular, but sometimes nearly clear. The cells contained granules of golden brown pigment as did the normal tubular epithelium. No mitotic figures were found.

Brain. The cytologic changes in the brain were essentially of two types, although variants of these occurred. The rather sharply defined nodules in the paraventricular regions and in the right frontal lobe differed chiefly in the degree of activity. They consisted of dense interlaced and whorled bundles of glial cells (Figs. 5 and 6), a majority of which were spongioblasts of unipolar or bipolar types (Fig. 7). Many large, bizarre and malformed cells of astrocytic type were present also (Fig. 8). These were frequently collected into groups and were particularly numerous about the periphery of the tumor of the frontal lobe. The cells in the paraventricular nodules tended to be more mature and no mitotic figures were found, whereas mitotic figures were easily found within the tumor of the frontal lobe.

The well defined nodules were well vascularized, the frontal tumor showing numerous engorged, thin-walled, branching, vascular channels of embryonal pattern. Foci of calcification were common and the transition from early degenerative changes to complete calcification could be traced in many of the enlarged misshapen cells. Nerve fibers and neurofibrils were demonstrable throughout the tumor nodules (Fig. 9). In the nodules and sclerotic patches myelination was generally poor and often practically absent.

In the tuberous nodules elsewhere there was gliosis consisting of

fully differentiated cells of astrocytic variety associated with varying numbers of large, rounded, ovoid, stellate, or globular cells (Fig. 10). These showed degenerative changes of which displacement of the nucleus, poor staining quality, hyaline degeneration of the cytoplasm, and great variation in the size, arrangement, and distribution of the processes were the most conspicuous. Some of these were definitely ganglion cells, but many appeared to be of glial origin. Differentiation between glial cells and nerve cells, however, was impossible in many instances, even with the aid of special gold and silver staining methods. In the sclerotic nodules the cyto-architecture was invariably severely deranged. Occasional vessels in the striate bodies were surrounded by collars of neuroblasts which extended into the adventitial coats. Petechial hemorrhages, often perivascular, were present in the internal portions of the cerebral hemispheres.

Lungs. The lungs showed intense congestion, with areas of early pneumonic exudation of lobular distribution.

Discussion

Tuberous sclerosis and various tumors, notably cardiac rhabdomyomatous nodules, coexist so frequently that their relationship is surely more than fortuitous. This relationship strongly supports the theory concerning the histogenesis of these conditions, namely, that they are manifestations of a generalized developmental defect, probably due to defective cell potencies. It is for this reason that the term tuberous sclerosis complex has arisen. In the survey of 63 congenital "rhabdomyomas" of the heart compiled by Batchelor and Maun,[1] tuberous sclerosis was present in 32 cases, but they, as well as others, are of the opinion that the coexistence of these two lesions occurs in a much higher percentage of the cases. Of these 32, only 2 presented solitary cardiac tumors, thus emphasizing the greater tendency for multiple nodules to be associated with tuberous sclerosis.

Hueper [6] has called attention to the rarity of rhabdomyomatosis of the heart in the Negro. He described its occurrence in a Negro boy, and, of the 45 cases reported up to 1935, his was the only example in a Negro. These cardiac tumors, in contrast to congenital developmental disturbances in other organs, apparently never show malignant transformation.

Moolten,[10] Bielschowsky,[11] Globus, Strauss, and Selinsky,[12] and Globus,[12] and Ferraro and Doolittle [13] have called attention to the frequency with which actual neoplasms occur in the cerebral foci and take the form of a malignant mixed tumor (neurospongioblastoma). Globus,[14] in his discussion of primary neuro-ectodermal tumors of the

brain, described 12 cases of glioneuroma and 10 cases of spongioneuroblastoma, with 2 of the former and all of the latter revealing features which indicated their close relationship to either fully developed or abortive forms of tuberous sclerosis. In practically every case of the glioneuroma group there were various cellular accumulations or patterns which suggested remnants of different phases of embryonal brain development.

Globus [14] felt that the presence of both spongioblastic and neuroblastic derivatives gave support to the Cohnheim-Ribbert theory of origin of neoplasms in embryonal rests.

Various renal hamartomatous lesions are present in more than half of the patients having the tuberous sclerosis complex.[10] These lesions generally show an organoid structure, and true neoplasms are rare. The collections of abnormal tubules in the present case certainly suggest a hamartial defect rather than a true neoplasm.

Conclusion

A premature Negro infant showed well developed lesions of the tuberous sclerosis complex. The changes in the brain were far advanced and at least one of the nodules showed a truly neoplastic transformation (neurospongioblastoma). The cardiac tumors were of the type most commonly referred to as "congenital rhabdomyoma." This is the 64th example of that condition to be recorded.

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DESCRIPTION OF PLATES

Plate 33

Fig. 1. Cross section of the brain showing the tumor of the frontal lobe and nodular masses projecting into the ventricle.

Fig. 2. Rhabdomyomatous nodule projecting from the apex of the heart.

Fig. 3. Large, clear, granular and vacuolated cells of the cardiac tumor, including spider cells. Pollak's modification of the trichrome stain. × 365.

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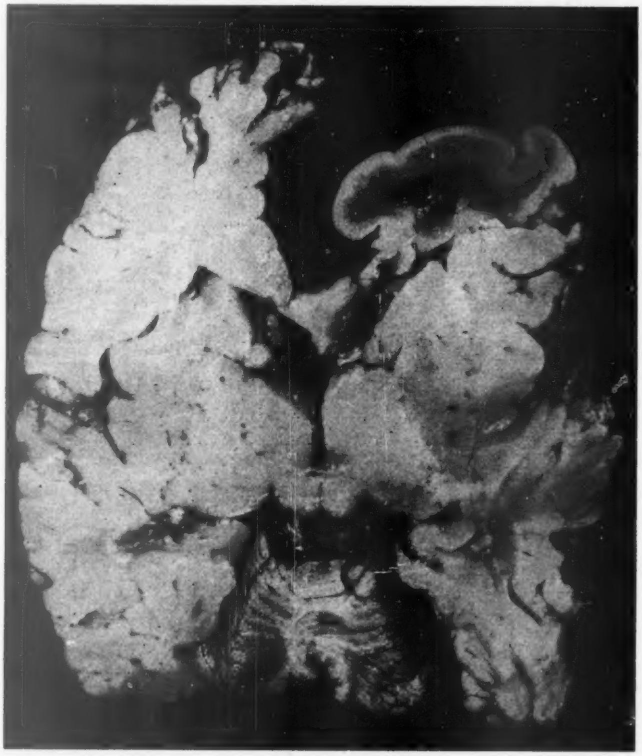

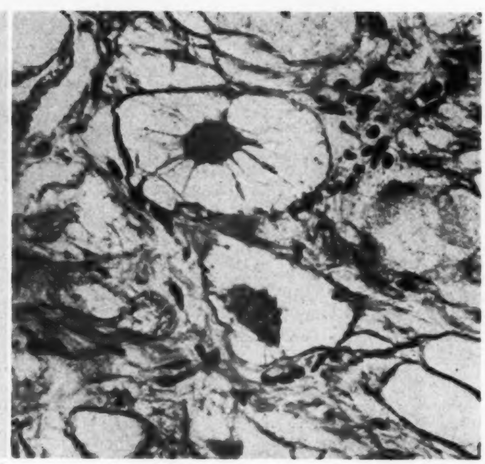

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Pratt-Thomas Tuberous Sclerosis

PLATE 34

FIG. 4. Dilated renal tubular structures lined by layers of cuboidal and columnar epithelium. Hematoxylin and eosin stain. × 115.

FIG. 5. Interlacing bundles of glial fiber in a ventricular nodule. Globus' modification of the gold sublimate stain. × 115.

FIG. 6. The tumor of the frontal lobe. Hematoxylin and eosin stain. × 115.

FIG. 7. Partially differentiated spongioblasts in the tumor of the frontal lobe. Globus' modification of the gold sublimate method. × 385.

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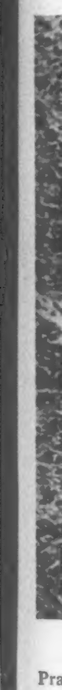

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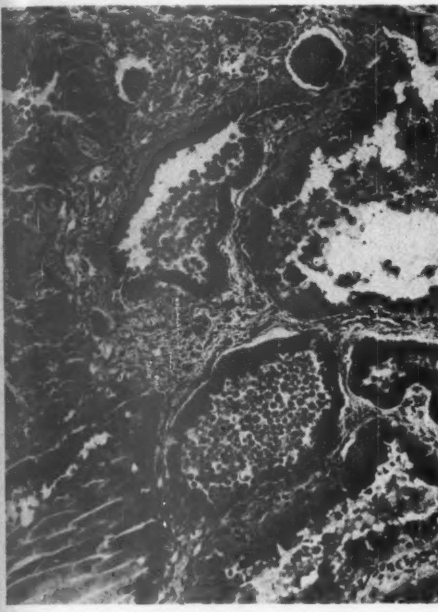

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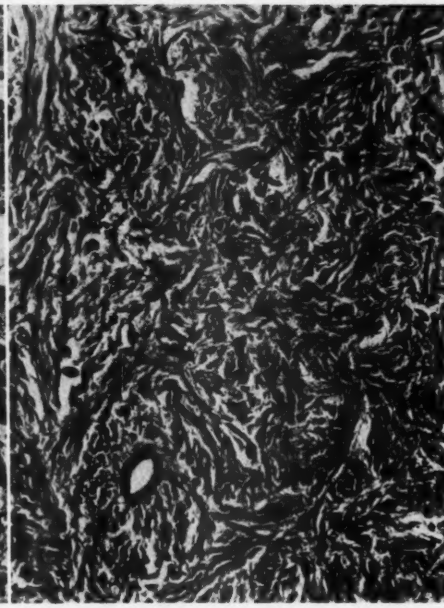

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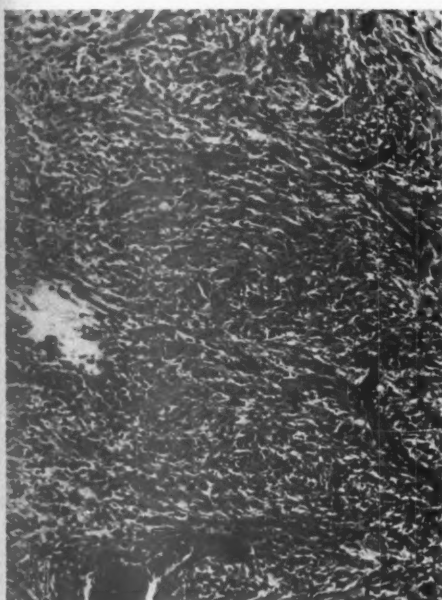

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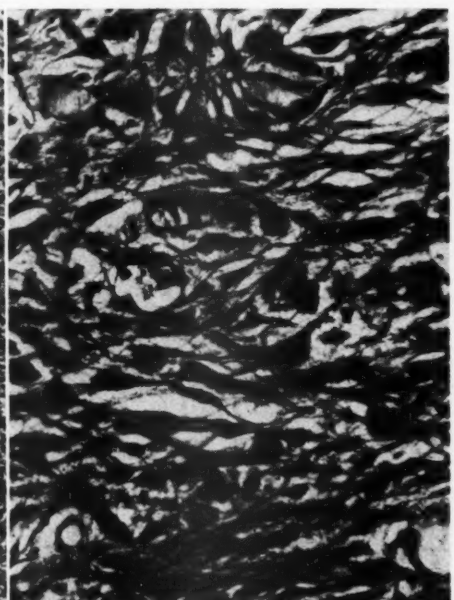

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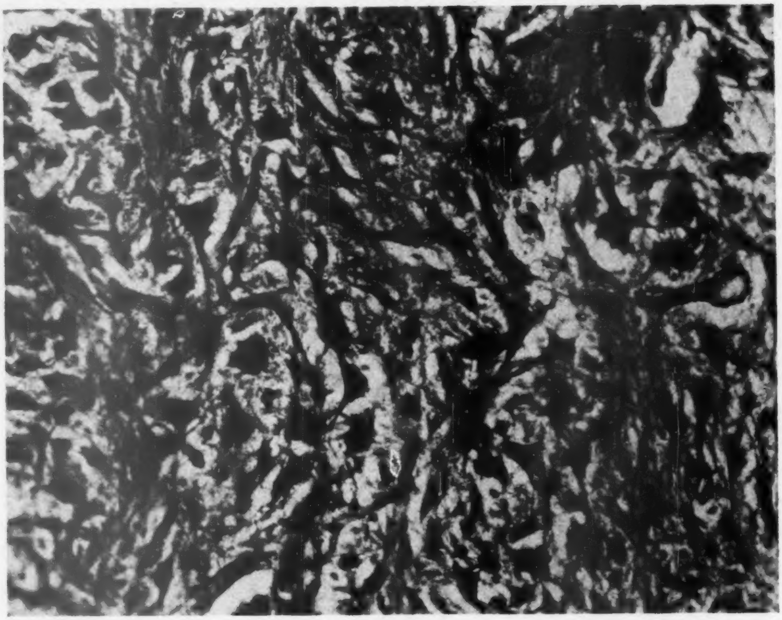
PLATE 35

FIG. 8. Bizzare, atypical glial elements in the periphery of ventricular nodules. Globus' modification of the gold sublimate method. × 350.

FIG. 9. Nerve fibrils among abnormal misshapen cells of the tumor of the frontal lobe. Bodian's method. × 600.

FIG. 10. Large, degenerating balloon-shaped cells in sclerotic nodules. Cresyl violet stain. × 600.



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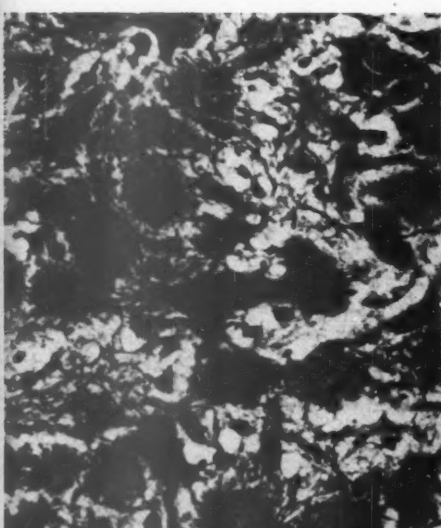

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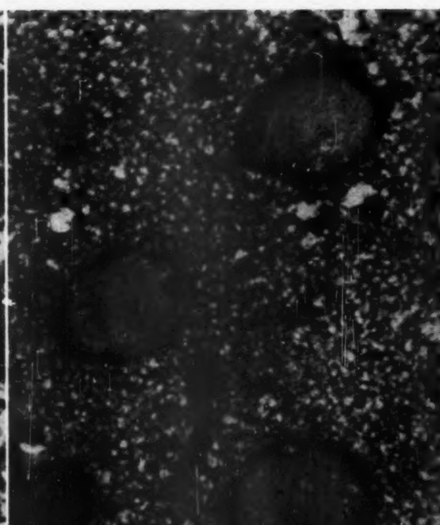

10

THE PATHOGENESIS OF POLYCYSTIC LIVERS

Reconstructions of Cystic Elements in Two Cases *

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The occurrence of congenital polycystic disease of the liver has been known and studied for a long time and the cause of the lesions has been the subject of much speculation. In addition to numerous case reports, reviews of the literature and discussions of the problem of etiology may be found in the papers of Kahlden,[1] Still,[2] Moschcowitz,[3] Bunting,[4] Vorpahl,[5] Meyenburg,[6,7] Sears,[8] Teuscher,[9] Wackerle,[10] Delore and Croizat,[11,12] Rümler,[13] Lutembacher,[14] Baccarini,[15] and Monserrat and Latienda.[16]

In those papers, it is clear that the cystic lesions have been thoroughly studied microscopically, often in serial sections, but they have not been evaluated by means of reconstructions. However, since it may rightly be assumed that the origin and mode of development of cystic lesions in all organs are governed by the same principles and since studies of models from polycystic kidneys have been made, the theories which have been proposed for the etiology of polycystic kidneys have been applied to polycystic disease of the liver. The kidney, furthermore, is probably the organ best suited for the study of this disease, since it is formed from two separated anlagen which subsequently unite and the individual nephrons are composed of elements from both anlagen. In the kidney, therefore, the character of developmental defects and the time of their occurrence should be much more easily demonstrated than in organs such as the liver, pancreas, and lungs, in which the proper formation of individual epithelial elements is not dependent upon the union of different anlagen. Nevertheless, information on these points has been only slowly accumulated. Consequently, the problem of polycystic disease of the liver will be more easily understood if the theories of origin of polycystic kidneys are briefly reviewed.

At first it was thought by Virchow,[17,18] and others that inflammatory lesions of the fetal kidney might disturb the developing nephrons sufficiently to cause obstruction and cystic dilatation. This theory at present is not in favor since inflammatory lesions are not always present and could scarcely be hereditary. Later it was suggested by

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Mutach,[19] Ribbert,[20] and others that failure of union of the two anlagen might explain these lesions. Although attractive by virtue of its simplicity, this theory is untenable since it does not explain why polycystic disease occurs in organs in which the union of two separate anlagen does not occur and since it has been shown recently [21, 22] that the two anlagen in all probability do unite. Because of the large size of many cysts and the occasional finding of undifferentiated masses of epithelial cells, it was proposed by Brigidi and Severi,[23] and others that the lesions were truly neoplastic. There seems little basis for this argument since the cysts show no evidence of rapid proliferation and the great majority are lined by only a single layer of epithelium. Much more logically, Albrecht [24] suggested the term "Hamartome" for these lesions, which impressed him as being an improper proliferation of tissue as a consequence of faulty development. However, recently, doubt has been cast upon the fundamental importance of excessive proliferation of epithelium,[22] since in one case segmentation of nephrons occurred without excessive dilatation or proliferation of epithelium. Important contributions to the subject recently were made by Kampmeier,[25-28] and McKenna and Kampmeier,[29,30] These workers demonstrated that the first generations of nephrons in the metanephros are normally provisional and often persist for short periods in fetal life as small, isolated cysts. They concluded that persistence of these segments as cysts might explain the occurrence of polycystic disease. More recently, however, Norris and Herman [22] found in one of their cases that virtually all of the nephrons were segmented and cystic and that the total number of nephrons was approximately normal for a newborn child. By means of serial sections and reconstructions, 4 cases were studied, 2 of which were newborn infants and 2 adults. In the 2 infants, normally formed but isolated glomeruli without marked capsular dilatation were found in the cortex, and blindly-ending but undilated collecting ducts emptying into the calyces were found in the papillae. Between the two zones were cysts and undilated segments of nephrons. It was concluded, first, that for the glomeruli to be so perfectly formed, continuity between the nephrogenic anlage and ureteric anlage must first have been established and have been followed by segmentation and cystic dilatation of the detached elements and, second, that excessive epithelial proliferation was not necessarily responsible for the lesions. It was also pointed out from the literature that the mesonephros normally degenerates by a process of segmentation as do the early generations of nephrons in the metanephros. It was suggested, therefore, that polycystic disease of the kidneys is in reality an extension of this normal process of degeneration to include some or all of the later generations of nephrons.

To determine whether this last theory is also applicable to polycystic disease in general, study of other cystic organs is essential. For this purpose, we are reporting descriptions of cystic livers, together with reconstructions of some of the lesions, from the 2 infants previously reported by Norris and Herman.[22]

Materials and Methods

Case numbers 1 and 2 of the present report correspond to the case numbers given before by Norris and Herman.[22] Briefly, case 1 was that of a full-term male infant in whom greatly enlarged polycystic kidneys and liver so embarrassed respirations that he died 20 minutes after birth. Case 2 was that of a full-term female infant who developed a hemorrhagic diathesis, jaundice, and evidence of renal insufficiency and died 24 days after birth. Following delivery, sixth digits of both hands were amputated. Relatively undilated cystic lesions of the kidneys, liver, and pancreas were found at autopsy. Since in the previous paper the clinical histories, anatomic diagnoses, and detailed findings in the kidneys were presented, only the gross and microscopic lesions of the livers will be described at this time.

All tissue was fixed either in Kaiserling's or Regaud's solutions. Blocks were embedded in paraffin and sections were stained with Delafield's hematoxylin and eosin. Sections for ordinary study were cut at 5 μ. From each liver, several hundred serial sections, 15 μ in thickness, were cut from each of four blocks about 2 cm. on a side. The cystic lesions and distorted bile ducts were traced and studied microscopically through these sections. Reconstructions were accomplished by the method previously described.[21]

Observations

Case 1

Gross Examination. The liver of case 1 weighed 270 gm. and, in spite of the enlargement, had distinctly normal contours. There were distinct right and left lobes. The gallbladder was partially distended with thick, dark green bile and, except for post-mortem changes of the mucosa, was normal. The cystic duct, extrahepatic bile ducts, and common duct were also patent and normal. On section, the parenchyma was bloody throughout. The lobular architecture was evident but was not as distinct as is normal. In the periphery of the lobules highly irregular cystic dilatations were seen averaging about 3 mm. in diameter. There were no large cysts nor any focal lesions.

Microscopic Examination. The lobular architecture of the liver was not distorted and branches of the hepatic veins were situated normally in the center of each lobule. The parenchymal liver cells showed no

lesions, but the sinusoids were slightly dilated and large areas of erythropoiesis and myelopoiesis were present. The periportal areas, however, were greatly distorted by marked cystic dilatations of the intrahepatic bile ducts which, as seen in low-power fields (Fig. 3), completely encircled cords of loose connective tissue containing branches of the hepatic artery and portal vein. These cysts were lined by single layers of cuboidal or low-columnar epithelium. The cytoplasm of these cells was usually clear and the nuclei were vesicular. There was no evidence of active proliferation. Under higher power, about the periphery of these cystic areas were seen numerous highly distorted bile ducts, lined by epithelium of similar character, which were not markedly dilated (Fig. 4). These, also, had a conspicuous tendency to encircle the branches of the portal vein and hepatic artery. The connective tissue surrounding these ducts was loose and in many areas cellular elements of the foci of hematopoiesis were migrating through the epithelium to the lumina of the ducts. Nearly all of the cystic bile ducts contained erythrocytes and casts of both hemoglobin and bile. The small bile canaliculi, however, were not dilated and did not contain casts.

In serial sections, as can be seen in the model (Fig. 1), the encirclement of the hepatic arteries and portal veins by the greatly dilated bile ducts was also readily demonstrated. The cystic ducts were highly irregular in contour and there were numerous large outpocketings. Anastomoses were numerous and, in many places, undilated but distorted ducts formed continuations and branches of the cystic ducts. Although many of the outpocketings or branches were blindly-ending, it was not demonstrated that any of the large dilated ducts were completely isolated as cysts. The courses of the hepatic arteries and portal veins were generally straight and uninterrupted although there was considerable irregularity of the contours of these vessels.

Case 2

Gross Examination. The liver of case 2, which weighed 220 gm., was normally formed and had distinct right and left lobes. The gallbladder contained a small amount of yellow bile and, except for post-mortem changes of the mucosa, was normal. The cystic duct, extrahepatic bile ducts, and common duct were likewise patent and normal. On section, the lobular architecture of the liver was grossly normal, the intrahepatic bile ducts were not dilated, and no focal lesions were seen.

Microscopic Examination. In low-power fields, the lobules of the liver were generally normal in contour. The central veins were not

remarkable and the parenchymal cells and sinusoids showed no lesions. Many of the branches of the portal vein, however, appeared dilated and were surrounded by larger numbers of bile ducts than is normal (Fig. 5). Under higher power, these ducts, although not conspicuously dilated, were highly irregular in contour, appeared more numerous than is normal and were grouped around the branches of the portal vein and hepatic artery (Fig. 6). They resembled the nondilated ducts in case 1 (Fig. 4). Individually, the lining cells were low-columnar or cuboidal. The cytoplasm was clear and colorless and the nuclei were vesicular. There was no evidence of rapid cellular proliferation. Many of the small bile canaliculi and some of the small bile ducts were slightly distended with casts of inspissated bile. There was no extramedullary hematopoiesis.

In serial sections, irregularity and distortion of individual bile ducts were marked. As can be seen in the reconstruction (Fig. 2), the ducts sometimes were narrow and sometimes were focally dilated. Small and large segments of ducts were often isolated as cystic structures without significant enlargement or dilatation when compared with the adjacent unsegmented ducts. Many of these isolated segments were in a direct line with branches of biliary ducts which were not segmented. This observation suggests that these segments were previously in continuity with the bile ducts and secondarily became isolated as nondilated cysts. Although the branches of the hepatic artery were generally straight, the branches of the portal vein were often distorted. No isolated segments of veins were identified.

Discussion

In the cases presented, the conspicuous lesions were those of the small intrahepatic bile ducts. The ducts in both cases were highly irregular in contour and diameter and did not conform to a regular pattern. The number of ducts in the periportal areas appeared to be greater than is normal. This was especially true in case 2, as can be seen in the model (Fig. 2). Anastomoses in both cases were numerous. In case 1, many of the ducts were not dilated, but in nearly all of the periportal areas there were large cystic ducts which in places completely encircled the hepatic artery and portal vein. None of these, however, were demonstrated as being isolated, blindly-ending cysts. By contrast, in case 2 none of the ducts were greatly dilated but many small and large segments were isolated and were blindly-ending in both directions. That the flow of bile, elaborated by the parenchymal epithelial cells, was obstructed in many places was shown by jaundice and by bile casts in the canaliculi. In case 1, however, there was no jaundice and the

canaliculi did not contain bile casts, so that biliary obstruction was not demonstrated.

It is surprising that in case 1 the large cystic ducts were not found isolated as cysts. If these ducts were not segmented, as the lack of obstructive jaundice also indicates, then it may be argued that cystic dilatation may occur before segmentation and isolation of these elements as cysts. In case 2, by contrast, segmentation occurred without cystic dilatation. On the other hand, in the kidneys of case 1 similar large cystic dilatations were shown definitely to be isolated as cysts. In the latter, there were also numerous anastomoses. It is quite possible, therefore, that similar anastomoses in the liver obscured a previous tendency to segmentation and isolation of cysts.

In the kidneys, although the method for estimating the number of nephrons was admittedly crude, it was not demonstrated that there was an overproduction of elements. In the livers, more than the usual number of bile ducts appeared to be present in the periportal areas. Theoretically, an excessive production of elements persisting until birth can occur, since early generations of nephrons are normally provisional as are many of the small intrahepatic bile ducts. The association of polydactylism with polycystic disease also suggests this possibility. That an overproduction of elements is significant in the pathogenesis of polycystic disease has been previously suggested and has been used as the basis for the assumption that abnormal proliferation of epithelial elements is the fundamental lesion of the disease.[13,23,24] However, the liver differs from the kidney in that bile ducts can regenerate in various diseases whereas nephrons, after birth, at least, do not have this potentiality. Moreover, anastomoses among small bile ducts are the rule but do not occur normally among nephrons. Consequently, so far as the present cases are concerned, preceding abnormalities of the developing bile ducts might stimulate the production of excessive numbers of elements. The apparent overproduction of bile ducts would then be the result of the lesions rather than the cause of them.

Before discussing further the significance of the cystic lesions which have been described and illustrated in these cases, it is important to review the normal development of the liver. According to Lewis,[31] the anlage of the liver is a median ventral outgrowth of the entodermal tube. Cords of epithelial cells proliferate distally and are later separated from the gut by a short, solid stem. Eventually this stem becomes canalized to form the common bile duct. Meanwhile the trabeculae and cords of proliferating epithelial cells indent the lumina of the omphalomesenteric veins which grow out between the cords to invest

and surround them with endothelium. The right omphalomesenteric vein later becomes the portal vein and the hepatic vein is essentially the persistent outlet of this right omphalomesenteric vein. The left umbilical vein also sends branches to the liver and becomes the round ligament after birth. For a period in embryonic development, the hepatic vein is connected with the portal and umbilical veins by a large blood sinus within the liver, the ductus venosus, which disappears before birth by subdividing into the sinusoidal circulation. Meanwhile, branches of the hepatic artery are proliferating along branches of the hepatic ducts which are developing in continuity with the common duct. The intrahepatic ducts, however, do not proliferate extensively until after branches of the portal vein are formed and generally follow the course of these branches. Between the embryonic stages of 10 and 23 mm., segments of blindly-ending ducts may be observed. These blend with the hepatic trabeculae, and it is not until the stage of about 23 mm. that proliferation of the duct system is active. These ducts form a plexus in the periportal mesenchyma. Anastomoses which are numerous at first are fewer at the time of birth, although fluid injected into one hepatic duct will be returned by way of the other.

From these facts concerning the development of the normal liver it is possible to date roughly the origin of the lesions in the present cases. Since intrahepatic bile ducts do not appear until the embryo is 10 mm. in length and do not proliferate actively until about 23 mm., 10 to 23 mm. is the earliest stage at which the lesions of the ducts could begin. By this time, however, the lobules are beginning to differentiate and branches of the portal vein and hepatic artery are already appearing in the perilobular mesenchyma. It is quite possible, therefore, that pathologic changes of the ducts did commence at this stage. The encirclement of branches of the portal vein and hepatic artery by dilated ducts in case 1 indicates progressive enlargement of the ducts after this initial stage and it is altogether likely that the development of the lesions in both cases was continuous but gradual.

The gross structure of the livers in both of the present cases was remarkably normal. The gallbladders were normally formed and there was no dilatation or atresia of the common bile ducts. Both grossly and microscopically, the lobular pattern was normal. The central veins, sinusoids, and parenchymal epithelial cells showed no lesions. Although there was some distortion and irregularity of the branches of the portal vein and hepatic artery, they were normally situated in the perilobular connective tissue. In both cases, therefore, the principal lesions were confined to the small intrahepatic bile ducts and were of such a nature that the normal development of the other elements of

the liver and biliary system was not prevented or significantly altered. This observation is also in agreement with the fact that the structural development of the cystic kidneys in those cases was likewise normal.

If these lesions were caused by inflammation, of which there was no evidence, or by an unrestrained proliferation of the bile ducts, it is difficult to understand how the rest of the liver could be so normal. Likewise, since so many of the ducts were distorted or segmented, the occurrence of the lesions can hardly be explained by the persistence of the few normally provisional bile ducts in accordance with Kampmeier's theory that persistence of normally provisional nephrons accounts for the polycystic lesions of the kidney.

In view of these various considerations, therefore, it is proposed that, as for the kidneys, differentiation of the hepatic anlage was at first normal. Only after the appearance of the small intrahepatic bile ducts did cystic lesions begin. Whether the apparent overproduction of the bile ducts was primary or secondary is immaterial. Distortion, segmentation, and cystic dilatation of these elements occurred progressively while the normal differentiation of the rest of the liver was proceeding uninterruptedly. Fundamentally, this is a process of degeneration and is analogous to the sequence of anatomic changes which have been demonstrated in the kidneys. As segmentation of elements is the initial stage of resorption in the mesonephros and in the first generations of nephrons in the metanephros, so the early generations of bile ducts normally segment before complete degeneration and resorption. Consequently, it is believed that in polycystic disease of the liver many more of the small intrahepatic bile ducts than is normal are provisional and that persistence of these segments, which may progressively become dilated as cysts, explains the polycystic disease of the adult. The evidence presented confirms the previous hypothesis that polycystic disease in general is occasioned by an abnormal extension of a normal process of degeneration. The lesions may or may not be accompanied by an overproduction of elements and cystic dilatation may occur before or after segmentation. The familial incidence of the disease strongly suggests a hereditary defect.

Summary and Conclusions

As part of a study of the polycystic lesions in the livers of 2 infants, three-dimensional reconstructions of some of the elements were prepared.

The lesions, confined exclusively to the intrahepatic bile ducts, consisted of distortion, segmentation, and dilatation.

The normal development and differentiation of the rest of the liver and biliary tract was not prevented or significantly altered.

As is the case in polycystic kidneys, it was concluded that the lesions in the livers are essentially degenerative and are an abnormal extension of the process of resorption which occurs normally in the first generations of bile ducts.

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DESCRIPTION OF PLATES

Plate 36

Fig. 1. Case 1. Model of dilated cystic bile ducts. Anastomoses and blindly-ending projections are numerous. At the extreme left of the model, relatively nondilated branches of the duct are seen in cross section. At the extreme right, the dark tubular structures are branches of the portal vein which in this instance lie outside the duct. Encompassed by the two large cystic ducts are branches of the hepatic artery which are also tubular and less darkly shaded. In the center of the model, windows have been cut to illustrate the cystic folds of the duct and the interior location of the artery. The approximate vertical extent of the reconstruction in the liver was 0.64 cm.

Fig. 2. Case 2. Model of reconstructed elements. Branches of the hepatic artery (A) and portal vein (V) are generally straight but are intimately associated and frequently surrounded by the numerous, more lightly shaded bile ducts. The ducts show considerable variation in diameter and lack a regular pattern. Many branches end blindly. In the lower half of the model are several completely isolated globular segments of ducts as well as a minute circular segment overlying the vein. Just to the right of center, also overlying a vein, is a highly irregular, nondilated segment of duct. Elsewhere there are several more isolated minute segments. Many of the isolated segments appear to be detached continuations of nearby ducts. The approximate vertical extent of the reconstruction in the liver was 0.14 cm.

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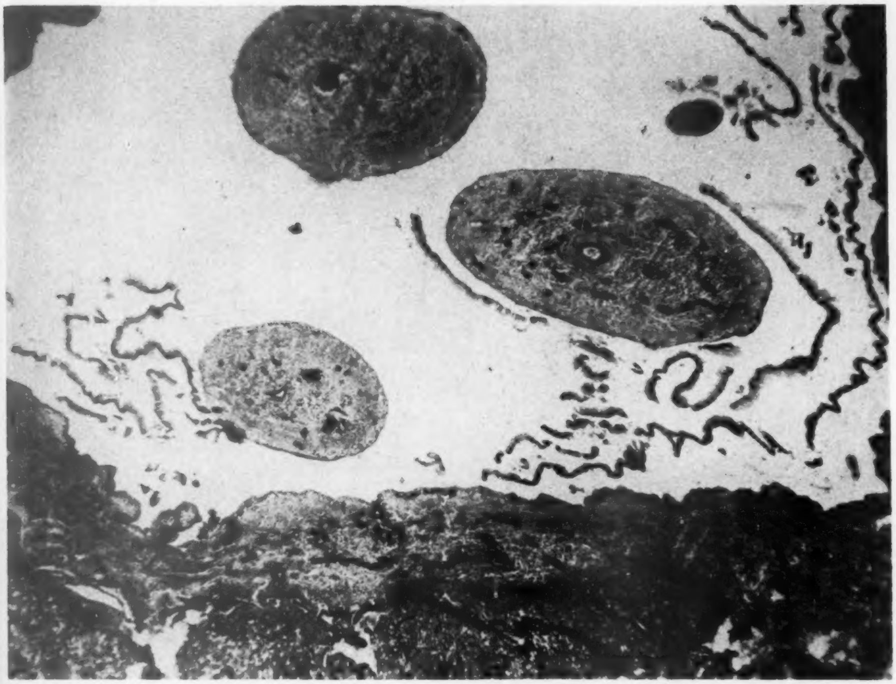
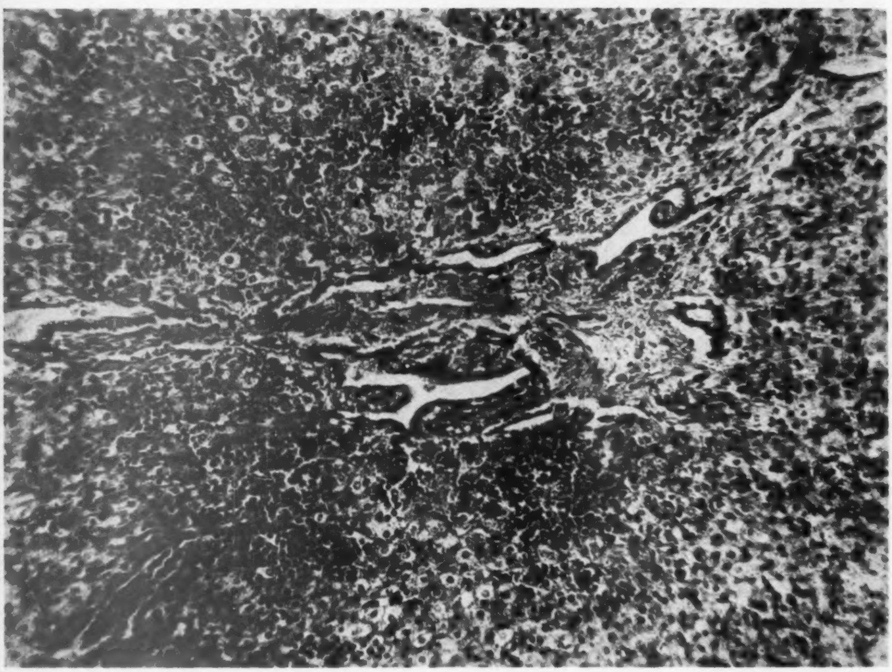
PLATE 37

FIG. 3. Case 1. Low-power photomicrograph showing a large cyst which is a cross section of a dilated bile duct. The lining columnar epithelium is generally detached. The circular cores of solid tissue within the lumen of the cyst are cross sections of trabeculae composed of periportal connective tissue which have been completely encircled and invested by the dilated duct. Embedded in the fibrous stroma are branches of the hepatic artery and portal vein. Hematoxylin and eosin stain. × 45.

FIG. 4. Case 1. Higher-power illustration of the relatively nondilated but highly irregular and distorted bile ducts about the periphery of one of the dilated cystic lesions. The number of ducts is greater than is normal. Small branches of the hepatic artery and vein lie between the ducts in the center. Foci of hematopoiesis are evident among the cords of parenchymal epithelial cells. Hematoxylin and eosin stain. × 190.





Norris and Tyson Pathogenesis of Polycystic Livers

PLATE 38

FIG. 5. Case 2. Low-power photomicrograph showing a periportal space in the center and the relatively normal lobular architecture. The centrally located circular space is a branch of the portal vein. Branches of the hepatic artery are adjacent. The vessels are completely encircled by small, irregular bile ducts which appear more numerous than is normal. Hematoxylin and eosin stain. × 23.

FIG. 6. Case 2. Higher-power illustration of a periportal space. The marked distortion and irregularity without significant dilatation of the small bile ducts are evident. There are also slightly distorted branches of the portal vein. Of note is the similarity of these ducts to those in Figure 4. Hematoxylin and eosin stain. × 190.

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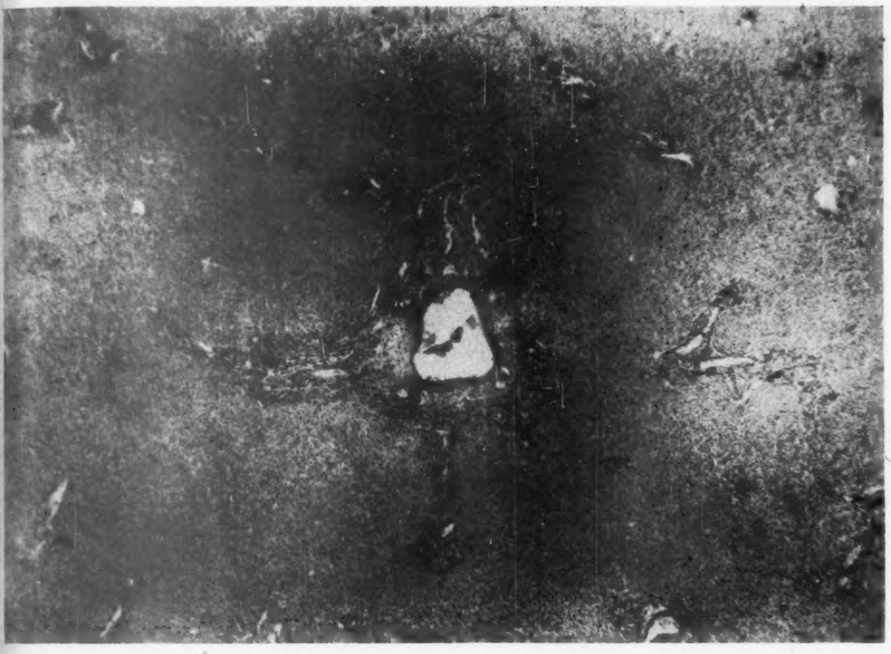

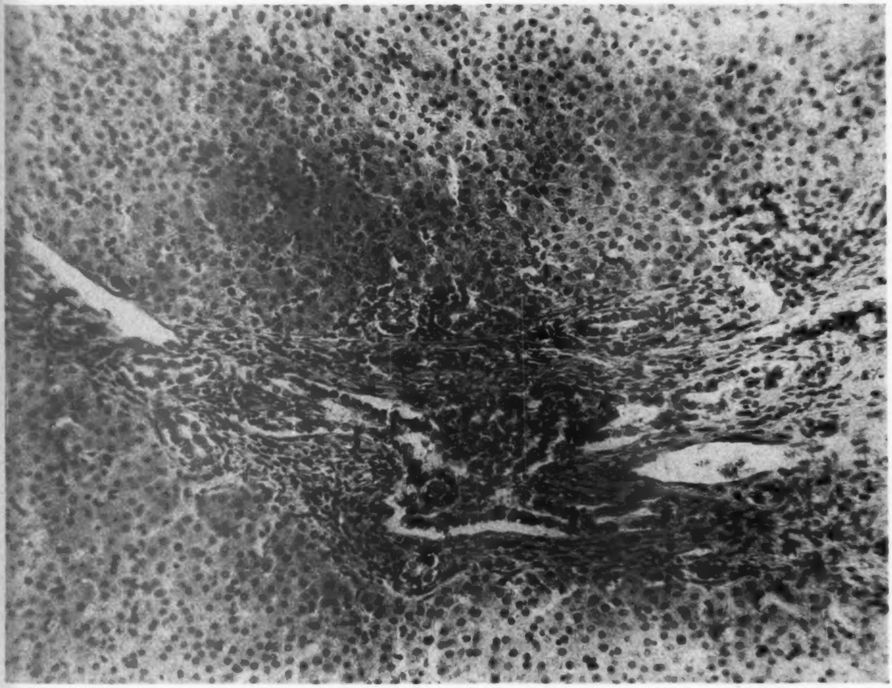

Norris and Tyson

Pathogenesis of Polycystic Livers

A HISTOLOGIC STUDY OF THE REACTION IN THE HAMSTER SPLEEN PRODUCED BY THE VIRUS OF COLORADO TICK FEVER *

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In a recent study [1] it was found that the blood serum from human cases of Colorado tick fever is infectious for the golden hamster. The present report is an outgrowth of that work and constitutes a study of the histologic reaction in the hamster spleen.

Methods of Study

Twenty-four normal hamsters were sacrificed and the various tissues were removed within 30 minutes of death and fixed for 5 hours in Zenker's fluid without the addition of acetic acid. Paraffin sections were stained with hematoxylin and eosin.

Sera from 6 typical human cases (all Caucasians) of Colorado tick fever were used to infect the initial groups of hamsters. Cases 11, 12, 13, and 14 are natural instances of the disease (see Table I for histories, physical and hematologic findings). Cases 34 and 39 resulted from experimental infections and have been previously reported.[1]

Four days after the 6 initial hamster groups were injected intraperitoneally with 0.5 cc. of serum from each of the 6 human cases, the animals were anesthetized and bled as previously described.[2] A small portion of the blood was oxalated for a study of the white blood cells and the remainder allowed to clot. The pooled serum from some of the hamsters was used to inoculate new groups of animals according to the sequence of transfers shown in Text-Figure 1.

The inoculated animals were killed on the fourth day, autopsied within 30 minutes of death, and the tissues prepared in the same manner as those from the normal animals.

Since no constant nor significant lesions were found in organs other than the spleen in a comparison of the central nervous system, bone marrow, spleen, liver, kidneys, gonads, heart and lungs of the normal animals and of those of the first experimental groups, this study was limited to the spleen.

Description of Normal Hamster Spleen

The normal hamster spleen is somewhat variable in size in animals of equal age and weight. It has a thin fibrous capsule and trabeculae, with the white pulp distributed along the arterioles. The red pulp

* Received for publication, March 14, 1946.

TABLE I

History, Physical and Hematologic Findings in Natural Cases of Colorado Tick Fever Used to Inoculate Hamsters

Case number	Age	Sex	Tick bite	Incubation (days)	Chilly sensations	Headache	Backache	Muscle and joint pain	Anorexia	Deep ocular pain	Photophobia	First attack (days)	Remission (days)	Second attack (days)	Characteristic fever	Days after onset of symptoms	Leukocytes in thousands per cmm.	Band forms (per cent)	Segmented forms (per cent)	Lymphocytes (per cent)	Monocytes (per cent)	Eosinophils (per cent)	Basophils (per cent)
11	7	M	+	4	+	+	+	+		+	+	2	2	2	+	6	2.25	11	17	63	9	0	0
12	23	M	+	6	+	+	+	+			+	3	2	2	+	1	3.55	17	46	29	8	0	0
																3	2.80	16	22	53	7	1	1
																5	1.80	29	37	29	5	0	0
																7	2.00	15	17	63	4	1	0
																10	4.05	10	32	54	3	1	0
13	65	M	+	3	+	+	+	+				2	1	1	+	4	4.35	8	42	45	5	0	0
14	12	M	+	5		+	+		+		+	2	1	2	+	5	1.50	29	21	39	11	0	0
																6	3.40	20	16	54	9	0	1
																7	3.85	17	9	67	6	0	1

consists of sinusoids and pulp spaces filled with blood, with groups of lymphoid cells scattered through it. In some animals these cells are present in large numbers and in a more or less diffuse arrangement. The white pulp consists of lymph follicles in which germinal centers are not well defined but with scattered large mononuclear cells with abundant pale cytoplasm interspersed with the smaller darkly staining lymphoid cells. At the periphery of the follicle there is a sharply defined, circular margin beyond which there may be several layers of somewhat larger and less darkly staining lymphoid cells (Fig. 1). The center of the follicle may normally contain deposits of brown granular pigment within mononuclear cells, and an occasional giant cell is found in the same region.

To determine whether normal serum had any effect on the spleen, 8 hamsters were each injected intraperitoneally with 0.5 cc. of normal human serum and 9 animals were injected in the same manner with normal hamster serum. Four days later the animals were sacrificed. Histologic study of the spleens revealed no change.

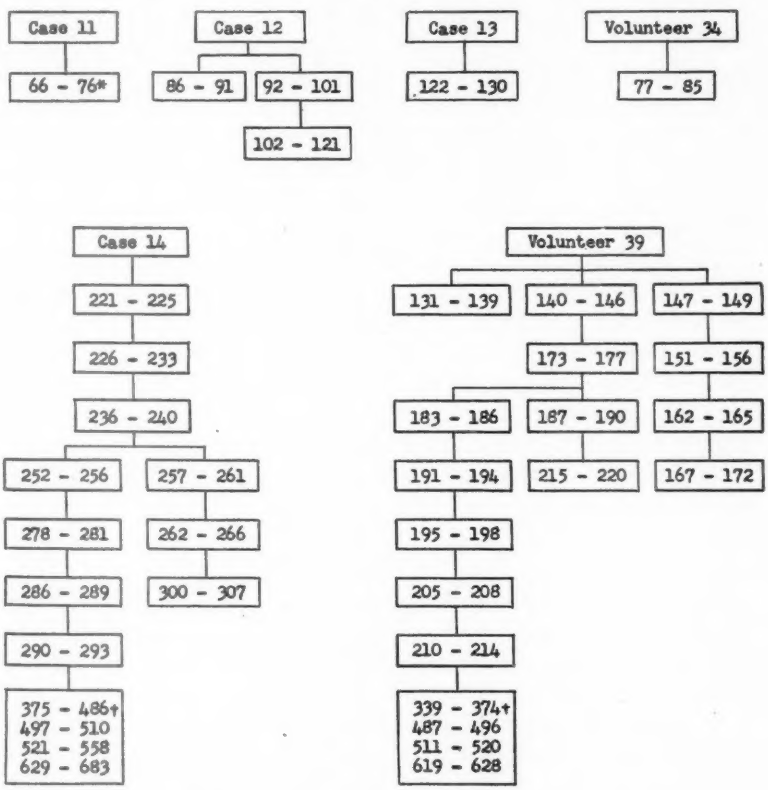
DESCRIPTION OF INFECTED HAMSTER SPLEEN

In the majority of spleens from infected animals, altera-

tions in the cellular type and arrangement of the follicular lymphoid tissue were found to be present, with variations in the extent but not in the type of reaction. There was an apparent reduction in the number of lymphoid cells in the central portion of the follicle, with the appearance

TEXT-FIGURE 1

Sequence of Transfers from Human Cases of Colorado Tick Fever Through Hamsters



* All numbers are inclusive.

† This block of numbers represents the animals used in determining the etiology of the disease.

throughout the follicle of large pale-staining mononuclear cells mingled with polymorphonuclear leukocytes and erythrocytes. The large mononuclear cells contained small, irregularly shaped masses of dark blue-staining material resembling fragmented nuclei. The periphery of the follicle showed a partial or complete disappearance of the normal well defined margin, which was replaced by a ragged border of mononuclear

cells, with occasional polymorphonuclear leukocytes and erythrocytes (Fig. 2). This reaction, as a whole, was graded ±, +, ++, or +++, according to its severity.

Following the usual custom in describing splenic phagocytes, the large mononuclear cells will be referred to as macrophages. Phagocytosis by the macrophages of whole lymphoid cells or of erythrocytes was not frequent. Congestion of the red pulp was not observed.

Staining methods employed in addition to hematoxylin and eosin included those of Giemsa and Gram, and Foot's modification of the Bielschowsky reticulum stain. With the Giemsa stain both eosinophilic and basophilic intracytoplasmic inclusions were seen in the macrophages. A large part of the included material was Gram-positive and evidently was composed of fragmented nuclei, presumably derived from phagocytosed lymphoid cells. Reticulum fibrils underwent some distortion but were not destroyed.

The possibility that these changes in the spleen were not caused by the virus of Colorado tick fever but were the result of some extraneous agent picked up in the serial passage of presumably normal hamster serum was eliminated in the following way. Serum from 5 healthy animals was pooled and 0.5 cc. was inoculated into each of 10 hamsters. They were bled on the fourth day and the procedure was repeated serially through 10 groups of approximately 10 animals each.[3] Sections from the spleen of each of the animals were studied. All were normal, as were the white blood cell counts.

Experimental Results

The data on white blood cell counts, presence of bodies in the lymphocytes as described in previous papers,[1,3] and the splenic reactions in the hamster groups used, whether or not they were included in the serial transfers, are shown in Table II.

In order to determine the duration and period of maximum intensity of the splenic reaction, two experiments were carried out. In the first, one group of hamsters was inoculated with serum from volunteer 39 and another group with hamster serum after having been passed serially through 6 groups of animals from a natural instance of the disease. At least one hamster was killed daily for 8 days in each of the 2 groups. In the second experiment, the same two strains were used. The first strain had been serially passaged through 18 and the second through 20 hamster groups. Eight to 10 animals were killed daily for 5 days.

The splenic reaction was definite on the second day, most pronounced on the third, and progressively less intense on the fourth and fifth

days. The average white blood cell count was lowest on the fourth day, at which time cytoplasmic bodies in lymphocytes also were most

TABLE II

Splenic Reactions, Mean White Blood Cell Counts, and the Presence of Cytoplasmic Bodies in Lymphocytes in Hamster Groups Inoculated with the Virus of Colorado Tick Fever

Animal numbers*	Source of material	Mean leukocytes in thousands per cmm.	Number of animals with cytoplasmic bodies in lymphocytes (100 cell hemogram)	Splenic reactions					
				0	±	+	++	+++	Not done
66– 76	Case 11	6.72 ± 2.22	0	6	3	1	0	1	
77– 85	Volunteer 34	5.37 ± 2.33	0	6	1	1	1	0	
86– 91	Case 12	6.39	0	6	0	0	0	0	
92–101	Case 12	5.03	0	10	0	0	0	0	
102–121	92–101	6.16	0	20	0	0	0	0	
86–121		5.89 ± 1.85							
122–130	Case 13	7.63 ± 0.79	4	0	2	4	2	1	
131–139	Volunteer 39	6.07	3	0	3	1	2	3	
140–146	Volunteer 39	4.16	3	1	2	1	1	2	
147–150	Volunteer 39	4.53	3						4
151–156	147–149	4.77	4	0	3	1	2	0	
157–166	151–156	6.01	4	0	1	4	1	4	
167–172	162–165	6.00	0	5	0	1	0	0	
173–182	140–146	4.49	3	0	5	4	1	0	
183–186	173–177	2.54	0	0	1	1	2	0	
187–190	173–177	5.86	0	1	1	2	0	0	
191–194	183–186	3.33	1	1	0	2	1	0	
195–204	191–194	3.80	1	0	0	5	2	3	
205–208	195–198	5.79	3	0	1	2	1	0	
209–214	205–208	4.86	3	3	1	1	1	0	
215–220	187–190	4.90	3	2	1	1	1	1	
131–220		4.87 ± 1.91							
221–225	Case 14	4.66	4	3	2	0	0	0	
226–235	221–225	5.01	5	1	4	1	3	0	1
236–251	226–233	5.84	2	4	5	4	2	0	1
252–256	236–240	5.36	3	0	1	3	1	0	
257–261	236–240	4.49	0	0	5	0	0	0	
262–277	257–261	3.61	0	2	2	5	4	3	
278–285	252–256	4.62	2	0	4	2	2	0	
286–289	278–281	4.04	0	0	0	2	2	0	
290–299	286–289	4.18	1	1	0	3	3	3	
300–307	262–266	4.24	3	0	3	3	1	1	
221–307		4.63 ± 1.86							
†131–307		4.75 ± 1.90							
‡339–486		4.77 ± 2.08	69	16	10	26	34	62	
487–510		5.43 ± 2.96	13	4	4	8	4	4	
511–558		5.34 ± 2.99	17	2	2	4	8	31	1
619–683		4.30 ± 1.73	16	14	4	12	17	18	
‡280 infected animals		4.81 ± 2.30							
§522 infected animals		4.76 ± 2.11							

* All numbers are inclusive.

† Since the difference between the means in each of the two major strains used is less than 1 standard deviation, the data for the two groups were combined.

‡ Animals 339–683 were used to determine the etiologic factor.

§ Since the difference between the means of animals 131–307 and those used in determining the etiologic factor is less than 2 standard deviations, they were combined.

commonly found. The splenic reactions are correlated with the total white blood cell counts and the presence of cytoplasmic bodies in Table III.

After the sixth or seventh day, the spleens of recovered animals were indistinguishable histologically from those of normal hamsters.

TABLE III

Duration of Splenic Reaction Correlated with the Mean White Blood Cell Count and Cytoplasmic Bodies in Lymphocytes

Days after inoculation	Animal numbers*	Mean leukocytes in thousands per cmm.	Number of animals with cytoplasmic bodies in lymphocytes (100 cell hemogram)	Splenic reactions				
				0	±	+	++	+++
1	308–310†	6.10	0	2	1	0	0	0
	684–691‡	6.99	0	8	0	0	0	0
	Both groups	6.75	0	10	1	0	0	0
2	692–699	6.27	0	0	0	1	6	1
	311–314	6.42	1	1	0	2	1	0
	Both groups	6.32	1	1	0	3	7	1
3	315–318	3.64	1	0	0	1	3	0
	700–709	4.84	1	0	0	0	1	9
	Both groups	4.49	2	0	0	1	4	9
4	710–719	3.84	4	0	0	4	3	3
	319–321	3.82	2	1	1	0	1	0
	Both groups	3.84	6	1	1	4	4	3
5	322–325	7.65	1	3	1	0	0	0
	720–727	5.29	0	3	0	4	1	0
	Both groups	6.15	1	6	1	4	1	0
6	326–329	4.43	0	4	0	0	0	0
7	330–333	10.15	0	4	0	0	0	0
8	334–336	4.16	0	2	0	1	0	0
9	337	6.60	0	1	0	0	0	0
10	338	7.50	0	1	0	0	0	0

* All numbers are inclusive.
† Numbers 308–338 represent the first experimental group.
‡ Numbers 684–727 represent the second experimental group.

DISCUSSION

No direct analogy can be found for this splenic reaction. Although gross swelling is not a prominent feature, the term acute splenic tumor may be applied to it. In man, many acute infectious diseases produce swelling of the spleen, with cellular reactions classified as either the red or gray type of acute splenic tumor. Comparison of the reaction in the hamster spleen in Colorado tick fever with the human spleen in various infections shows practically no resemblance to the gray type, and only a very general similarity to the red type.

Experimental tularemia in the hamster does not give a splenic picture such as we have observed.[4] It is impossible to say that the splenic reaction in the hamster with Colorado tick fever is pathognomonic of this disease. This decision must await further work on other diseases with these animals.

It will be seen that animals inoculated with serum from case 12 failed to show any deviation from normal in the histologic picture of the spleen. This is the only instance in which the hamster groups did not develop a characteristic splenic reaction as a result of inoculating serum from an individual who was considered to have a typical case of Colorado tick fever.

With the exception of the 36 animals inoculated with serum from case 12, only 72 of the 484 spleens studied failed to show a positive reaction in the inoculated animals. Sixteen animals (3 per cent) failed to show a white blood cell count under 6,000 cells per cmm., cytoplasmic bodies in the lymphocytes, or a positive splenic reaction. The splenic reaction is most likely to be negative in the original human-to-hamster transfer.

It must be pointed out that the results recorded in Table III constitute two separate experiments that possibly are not comparable. In the second experiment, the infectious agent had been passaged through many more hamster groups than in the earlier experiment.

Summary

1. A study of the spleens from hamsters infected with Colorado tick fever showed alterations in the cellular type and arrangement of the follicular lymphoid tissue as well as a partial or complete disappearance of the normal well defined follicular margin.

2. This reaction was observed in 79.3 per cent of 520 infected animals. These figures include the one instance of the disease that failed to show this reaction in a series of 36 animals.

3. Normal hamster serum passaged through 10 groups of animals failed to elicit these responses.

4. The splenic reaction was observed first on the second day following inoculation, reached its height on the third day, and continued through the fifth day.

We wish to thank the Misses Emma Martin, Marguerite Jenks, and Marguerite Gagliardi for preparing the large number of tissue sections used in this work.

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DESCRIPTION OF PLATE

PLATE 39

FIG. 1. Normal hamster spleen. × 140.

FIG. 2. Positive reaction (++) in hamster spleen. A central arteriole may be seen to the left of the center of the field. × 140.

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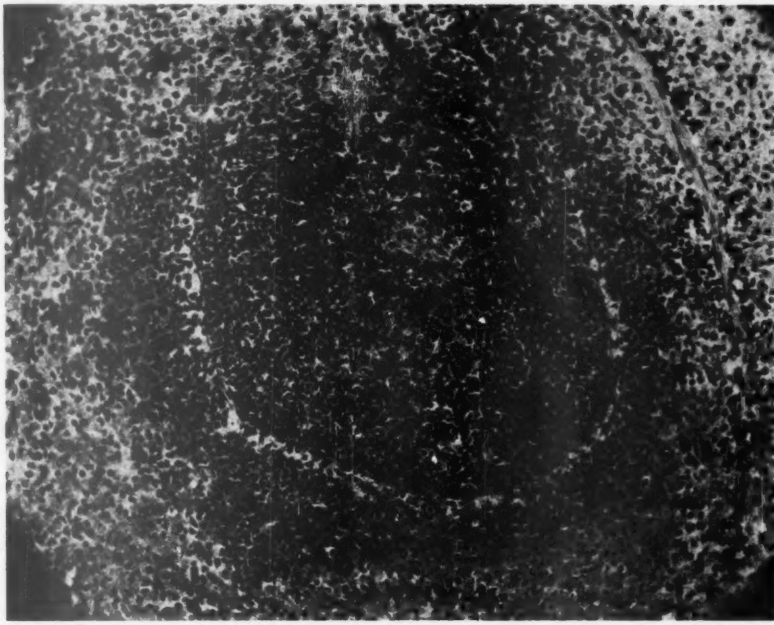

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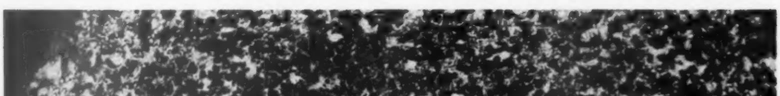

Black, Florio, and Stewart Hamster Spleen in Colorado Tick Fever

BLINDNESS IN DUCKS ACCOMPANYING HYPOGLYCEMIA

A Clinical and Pathological Study *

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In a recent study of hypoglycemia in ducks,[1] it was observed that birds with a marked reduction of blood glucose wandered purposelessly about the laboratory and frequently ran into objects. Furthermore, they did not react when a hand was moved near their eyes. A disturbance in vision also has been observed in ducks with a severe malarial infection. The latter birds had acute degenerative lesions in the optic nerve and brain tissue.[2] Clinical observations, the glucose levels of the blood, and the pathological lesions as observed in the central nervous system of ducks given insulin are reported in this paper.

Materials and Methods

The ducks were white Pekins, 4 to 6 weeks of age. They were given 30 units per kg. of both plain insulin, "Iletin," and protamine zinc insulin. The former preparation was given intravenously and the latter subcutaneously. Approximately 8 hours following the first injection of insulin, a second injection of 15 units of protamine zinc insulin per kg. of body weight was given. A third dose of protamine zinc insulin, 30 units per kg., was given approximately 16 hours following the second injection.

Blood for glucose determination was obtained from either the inner surface of the wing or the web of the foot. The determinations were made according to the Hagedorn-Jensen method as given by Peters and Van Slyke,[3] except that the blank was added to, instead of subtracting from, the titration values.

The birds used for pathological study were sacrificed by decapitation at intervals varying from 2 to 60 days. The brain and the eyes were removed from each bird while the spinal cord and ganglia were obtained from some of them. One-half of each tissue was put into Bouin's solution and the remainder into a 10 per cent solution of formalin. Paraffin sections were prepared from the Bouin-fixed tissues and they were stained with hematoxylin and eosin, thionin, Weil's myelin sheath stain, and ammonical silver hydroxide.

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Some of the ducks were given a 5.0 per cent solution of glucose intravenously and were fed by stomach tube following the development of neurological manifestations. This was done to obtain material for study of the pathological changes in the nervous system at varying intervals after recovery from the acute phase of hypoglycemia.

Experimental Findings

Thirteen ducks were given insulin in this experiment. A few of these birds were less active than normally 8 hours following the first injection. Blindness first appeared on the second experimental day, approximately 6 hours after the third injection of insulin. Each bird in this group was either partially or completely blind at this time. They were weak and usually sat together in a corner of the battery. They neither ate nor drank during this period. Usually the birds had convulsions on the third day; however, duck 1441 had a convulsion on the second day. Five ducks (1436, 1441, 1451, 1453, and 1454) were blind, or had convulsions, and were killed between the second and fourth day of the experiment.

Four of the ducks (1440, 1450, 1455, and 1456) were given glucose intravenously and food and water by stomach tube after they became blind (Table I). Duck 1455 had numerous convulsions on the 5th day, became moribund, and was killed. The other three ducks in this group recovered their sight by the 5th day and appeared normal at the time they were killed on the 17th, the 25th, and the 39th day of the experiment.

The glucose level of the blood was followed in three ducks (1436, 1440, and 1441) as shown in Table I. The normal level of blood sugar in these three birds was 188, 159, and 172 mg. per cent, respectively. The blood glucose was approximately 100 mg. per cent on the afternoon of the first day. On the afternoon of the second day, it was 57 and 74 mg. per cent in two birds, which were blind. One duck at this time had convulsions and died. On the third day the blood sugar varied between 50 and 66 mg. per cent. One of these ducks (1436) was killed on the third day. The third duck in this group (1440) was given glucose, food, and water on the third day of the experiment. The blood sugar was 197 mg. per cent on the morning of the fourth day. Vision had improved by this time and apparently it was normal on the fifth day following the first injection of insulin. This duck was killed on the 39th experimental day.

Four ducks (1539, 1540, 1541, and 1542) were given three injections of insulin at the same times as those given to the other ducks used in this study. Each bird was blind on the third day of the experiment.

During this time, duck 1539 had several convulsions and was killed on the third day. Duck 1542 was weak and had many convulsions during the first 4 days of the experiment. It was given glucose intravenously and food and water by stomach tube. Ducks 1540 and 1541 were blind on the third day; however, their sight returned by the fourth day.

The glucose level of the blood was followed in these 4 ducks. It was 68 mg. per cent in duck 1539 on the day it was having convulsions and was killed. The blood glucose in duck 1542 was only 84 and 77 mg. per cent on the third day, 57 and 56 mg. per cent on the fourth day, and 41 mg. per cent on the fifth day. By the eighth experimental day the glucose level was normal. This bird had many convulsions during this time and was given glucose intravenously. Sight was markedly impaired until the eighth day. The lowest glucose level was 90 mg. per cent in ducks 1540 and 1541. Sight returned in these two birds by the fourth day.

Ducks 1540, 1541, and 1542 were kept for 60 days. During this time they showed some incoordination of movement. One could not be sure that sight was impaired; however, at times their actions suggested some disturbance in vision.

There were no macroscopical lesions observed at autopsy in either the birds sacrificed during the early phase of hypoglycemia or in those killed 60 days later. Microscopically, however, the small blood vessels were dilated and filled with blood in all sections removed from the brains and spinal cords of ducks sacrificed during the first 5 days of the experiment. The endothelial cells were swollen and the perivascular spaces were enlarged. The tissue was loose and in areas it appeared reticulated. The nerve fibers were swollen. The retina was edematous. Many focal areas of degeneration of various sizes and shapes were present in the substantia alba and in the great fiber tracts throughout the diencephalon, brain stem, and cerebellum. These lesions were present in the myelinated fiber tracts and in the reticular formation. They were numerous in the optic tracts and in the optic nerves (Figs. 1 and 2). Many of the nerve cells in the brain and spinal cord were swollen and showed central chromatolysis. This change was especially pronounced in the cells of the brain stem and cerebellum.

The nerve fibers in the focal areas of degeneration were swollen and demyelinated, as shown in the myelin sheath preparations. The axis cylinders were refractory to silver impregnation and many were fragmented. The nerve and glial cells remaining in these focal areas were swollen, pale-staining, and frequently fragmented. The nerve cells and fibers in the peripheral nerves and ganglia showed only minimal changes.

TABLE I

Effect of Hyperinsulinism on Ducks

Exper. day	Ducks								
	1441	1451	1436	1453	1454	1455	1450	1456	1440
1	Weight, 1370 gm.; 9 a.m., b.s.,* 172; 9:30 a.m., insulin;† 1 p.m., b.s., 99; 4:30 p.m., insulin	Weight, 1320 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1480 gm.; 9 a.m., b.s., 188; 9:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1425 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1555 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1475 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1075 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1480 gm.; 9 a.m., b.s., 159; 9:30 a.m., insulin; 1 p.m., b.s., 95; 4:30 p.m., insulin
2	9 a.m., b.s., 108; 9 a.m., insulin; 2 p.m., convulsion, died	8:30 a.m., insulin; 3:30 p.m., blind; 3:15 p.m., killed	9 a.m., b.s., 124; 9 a.m., insulin; 2:30 p.m., blind; 4 p.m., b.s., 74	8:30 a.m., insulin; 4 p.m., impaired vision	8:30 a.m., insulin; 3:00 p.m., blind	8:30 a.m., insulin; 3 p.m., impaired vision	8:30 a.m., insulin; 3 p.m., blind	8:30 a.m., insulin; 3:00 p.m., blind	9 a.m., b.s., 146; 9 a.m., insulin; 2:30 p.m., blind; 4:30 p.m., b.s., 57
3			9 a.m., b.s., 59; 2 p.m., blind; 2:15 p.m., killed	10 a.m., blind, convulsion; 11 a.m., convulsion; 4 p.m., convulsion; 4:15 p.m., killed	8:30 a.m., blind; 6:00 p.m., blind	9 a.m., blind; 4 p.m., blind; convulsion, glucose‡	9 a.m., blind; 4 p.m., convulsion, glucose; 8 p.m., sight improving	8:30 a.m., blind; 3 p.m., impaired vision, glucose; 8 p.m., sight improving	9 a.m., b.s., 50; 11 a.m., glucose; 2:30 p.m., glucose; 4 p.m., b.s., 66; 4:30 p.m., glucose

4					8:30 a.m., blind; 2 p.m., blind; 2 p.m., killed	8:30 a.m., glucose, blind; 2 p.m., blind; 3 p.m., glucose; 8 p.m., blind	8 a.m., blind, glucose; 2 p.m., blind; 3 p.m., glucose	8 a.m., glucose; 9 a.m., normal; 2 p.m., normal	9 a.m., b.s., 197; 10 a.m., glucose, impaired vision; 4 p.m., b.s., 274
5						9:30 a.m., convulsion, glucose; 1 p.m., blind; 9 p.m., convulsion; 11 p.m., convulsion; 12 p.m., moribund, killed	1 p.m., impaired vision; 9 p.m., normal; Killed on 17th day	Normal; Killed on 25th day	Normal; Killed on 39th day

* b.s. = blood sugar in mg. per cent.

† On the morning of the first day, 30 units of straight insulin were given intravenously and 30 units of protamine insulin were given subcutaneously per kg. On the afternoon of the first day, 15 units of protamine insulin were given subcutaneously per kg. On the morning of the second day, 15 units of protamine insulin were given subcutaneously per kg.

‡ The glucose is a 5.0 per cent solution, usually 10 cc. given intravenously.

Histological studies of the nervous tissue from ducks sacrificed 17 days or longer following the injection of insulin showed small focal areas of degeneration in the fiber tracts similar to those observed in the acute phase except that they were "filled in" by nerve fibers and glial elements (Figs. 3 and 4). The nerve fibers were normal in size and the tissue was more compact than that observed during the acute phase of hypoglycemia. Only minimal alterations were present in the nerve cells at this time. No significant changes were observed in either the peripheral nerves or the ganglia.

Discussion

The ducks were blind on the second day following the injection of large amounts of insulin. At this time the glucose levels of the blood were markedly decreased. This disturbance in vision was only temporary since sight gradually returned following the return of the blood glucose to normal levels. Edema with separation of the nerve cells and fibers, and focal necrosis characterized the early changes in the brain, optic nerve, and retina of these ducks. After recovery from the hypoglycemia, these lesions markedly regressed, as indicated by the subsequent histological studies. A disturbance in coordination and vision accompanying hyperinsulinism has been observed in the pigeon [4] and the rabbit.[5]

The pathological changes observed in the brains of these ducks with hypoglycemia are similar to the lesions observed in ducks with a severe malarial infection.[2] It has been suggested elsewhere [6,7] that the pathological lesions occurring in the brains of ducks with malaria result from anoxia. In cerebral glycolysis both glucose and oxygen are essential. It appears, therefore, from the above experimental studies that a similar lesion may develop in the brains of ducks if either anoxia or hypoglycemia occurs. Himwich [8] has recently reviewed the phenomenon of cerebral glycolysis and has emphasized the fact that different parts of the brain glycolyze at different rates. It is suggested from this study that the optic nerve in the duck may be very susceptible to a lack of glucose.

The return of sight following the return of the glucose levels of the blood to normal indicates that the pathological change is reversible. The early lesions in the brain associated with hypoglycemia suggest that cellular degeneration is minimal while edema is marked. Therefore, with the elapse of time and the restoration of the metabolic processes to normal, the pathological processes regress. These lesions may not heal completely, as shown by the histological studies made on birds that survived the acute phase of glycemia. Residual damage has been observed in the brains of ducks following the acute manifestations

of malaria.[2] Kabat, Dennis, and Baker [9] also observed residual damage in dogs previously kept under anoxic conditions. It may be that the number of areas of degeneration are too few and their location in the brains of hypoglycemic ducks are such that clinical changes are not pronounced. It is more difficult, of course, to discern either a few or minimal neurological changes in the duck than in man. Although carbohydrate metabolism in the duck differs from that in man, it is of interest to know that pathological changes have been reported in human brains following the injection of large amounts of insulin.[10,11]

The sameness of the pathological lesions in the brains of hypoglycemic ducks and those kept under anoxic conditions is interesting when considered along with the study of Gellhorn, Ingraham, and Moldavsky [12] on the influence of hypoglycemia on the sensitivity of the central nervous system to oxygen want. They wrote:

> "If the theory .... is correct, that a lowering of the blood sugar reduces both the sugar and oxygen consumption of the brain, the rate of oxidation in the brain should be diminished to such an extent that a condition prevails comparable to that induced by inhalation of low oxygen tensions. If this were the case, lowering of the blood sugar during a mild degree of oxygen deficiency would induce symptoms of more severe anoxemia .... than would occur at normal blood sugar levels. This would mean, in other words, that as the blood sugar level progressively falls the increment of rise in the blood pressure from inhalation of a given oxygen tension would steadily increase. This indeed had proven to be the case."

The fact that oxidative processes in the brain are diminished in hypoglycemia appears to be a more significant factor in the production of the cerebral lesions than that they result only from the convulsions, as suggested by Grayzel.[13] Terplan [14] likewise does not believe that mechanical factors alone produce the type of cerebral lesions observed in hypoglycemia. The study of Glickman and Gellhorn [15] furthermore would suggest that the lesions observed in hypoglycemia are not the result of convulsions. These investigators pointed out the fact that low blood sugar interferes with the oxygenation of the central nervous system. Therefore, a mild degree of oxygen deficiency under conditions of low blood sugar leads to symptoms which are similar to those obtained at a normal blood sugar level only under conditions of extreme anoxia.

Summary

Ducks made hypoglycemic by the injection of large amounts of insulin will lose their sight. Their sight returns, however, with a return of the blood sugar to normal levels. Focal areas of edema and degeneration occur in the brain, optic nerves, and retina of these hypoglycemic birds. These lesions are similar to those observed in the brains of anoxic ducks, and apparently they result from a disturbance in cerebral glycolysis.

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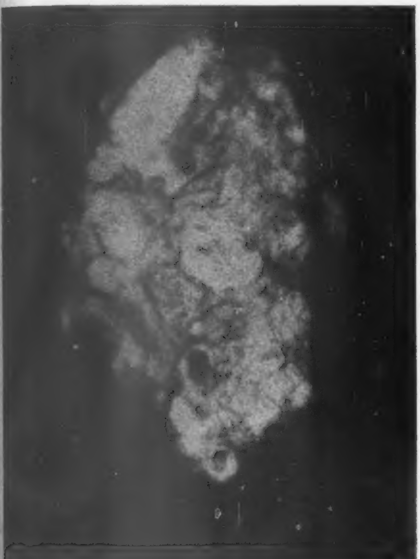
DESCRIPTION OF PLATE

Plate 40

Figs. 1 and 2. Many focal areas of edema and degeneration of nervous tissue occur in the optic nerve and tract, optic tectum, brain stem, and cerebellum of ducks given large quantities of insulin. At this time the birds are blind and the lesions are characterized by marked edema, degeneration of the nerve and of glial cells and fibers. Figure 1 is a section from the optic tectum of duck 1436, killed on the third experimental day. Figure 2 is a section from the optic nerve of duck 1453, killed on the third experimental day. Hematoxylin and eosin stain. × 440.

Figs. 3 and 4. The acute lesions apparently are reversible since the ducks recover their sight when the blood glucose returns to normal levels. The acute focal lesions gradually are filled in by nerve fibers and glial elements. This reparative process is not complete in all birds by the 60th day. Figure 3 is a section from the optic tectum of duck 1456, killed on the 25th experimental day. Figure 4 is a section from the optic nerve of duck 1440, killed on the 39th experimental day. Hematoxylin and eosin stain. × 440.



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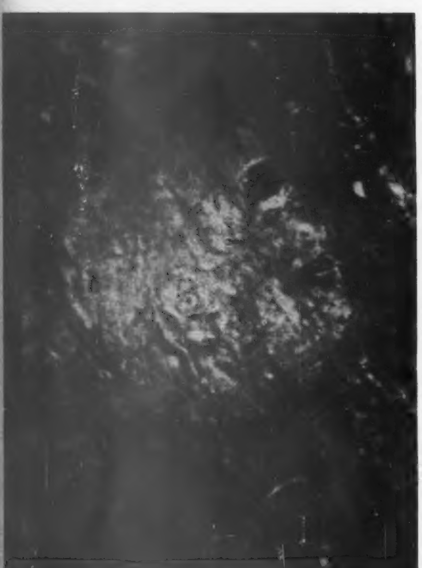

4

Rigdon and Fletcher		Blindness in Hypoglycemic Ducks

PLASMACYTOMA OF THE STOMACH

Report of a Case *

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Extramedullary plasma cell tumors are relatively uncommon. Those occurring in the gastrointestinal tract are extremely rare. Hellwig [1] recently reviewed 127 cases reported between 1905 and 1942, and added one of his own. Of these tumors, 63 arose in the upper respiratory tract, 47 in the conjunctiva, 4 in the lymph nodes, 2 each in the thyroid gland and the skin, and 1 each in the pleura, lacrymal gland, kidney, spermatic cord, vulva, and gastrointestinal tract.

No case of primary plasmacytoma of the stomach was found in a review of the literature.† Brown and Liber [2] cited the report by Vasiliu and Popa of a case with multiple ulcerated nodules in the mucosa of the stomach and intestines. These lesions were composed of plasma cells. Recently we found a plasmacytoma in a resected stomach, and the case was subsequently studied at autopsy.

Report of Case

The patient was a colored male farmer, 42 years old, whose chief complaint was "stomach trouble." He had been in good health until 7 years prior to admission. The first symptom was pain in the right lower quadrant gradually extending to the epigastrium. This was associated with frequent vomiting and inability to retain food. The pain was severe enough to require bed rest for a few weeks. Following this acute onset, the patient had slight, intermittent abdominal cramps for 6½ years. These were not severe enough to interfere with his work.

The patient experienced epigastric pain and vomited following breakfast 3 months prior to admission. This vomiting persisted, and was postprandial. Two tarry stools were observed by the patient 2 weeks prior to admission. Since his first attack he had been constipated; this was accentuated following his second attack. His average weight was 150 pounds, but at the time of admission it was only 105 pounds.

Laboratory Data. Complete pyloric obstruction was demonstrated roentgenologically. Roentgenograms of the heart, lungs, and long bones were normal. On two examinations the gastric contents were negative for free hydrochloric acid, and a large residuum was present. A Kahn test of the blood was negative. The red blood cell count was 4.4 million and the hemoglobin was 10.25 gm. The white blood cell count was 6,500, with 52 per cent polymorphonuclear leukocytes, 40 per cent lymphocytes, 4 per cent staff cells, 2 per cent juveniles, and 2 per cent eosinophils.

Clinical Course. A diagnosis of complete pyloric obstruction, due probably to a duodenal ulcer, was made, and an exploratory laparotomy was performed.

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† After this paper was submitted for publication, a similar case was reported by J. S. Couret (Extramedullary plasma cell tumor of the stomach. *Am. J. Clin. Path.*, 1946, 16, 213–218.

A smooth, firm annular mass, 3.0 cm. in width, was present in the wall of the stomach in the prepyloric region. It did not extend into the surrounding tissue. The regional lymph nodes varied from 1 to 2 cm. in diameter. They were soft and freely movable. No metastases were found in the viscera. A subtotal gastric resection was performed. The postoperative course was considered normal, and the patient was allowed to get out of bed on the 10th postoperative day. On the 14th day, while dressing to go home, he became weak, went to bed, and expired immediately.

Pathologic Examination of the Surgical Specimen

The surgical specimen consisted of a portion of stomach and duodenum which measured 15 by 13 by 2 cm. An annular ulcer, 9 by 5 cm., was present in the prepyloric region. The base of the ulcer was firm, irregular, gray, and 2 cm. thick. The edges were slightly rolled and hyperplastic. The uninvolved wall of the stomach measured 1 cm. in thickness. Seventeen lymph nodes were found attached to the serosal surface of the specimen, the largest measuring 2 by 1 cm. They were firm and discrete and the cut surface was gray and homogeneous.

The entire wall of the stomach was infiltrated at the site of the ulcer by large mononuclear cells. However, they did not involve the serosa. Many small mononuclear cells also were found. It was estimated that 80 per cent of the infiltrating cells were plasma cells.

The lymph nodes retained their normal architecture. The sinusoids were distended with fluid, plasma cells, and a few leukocytes. Large numbers of plasma cells were also found in the stroma. The perinodal fat was moderately infiltrated by plasma cells.

The individual plasma cells were for the most part typical. They were oval to round cells with abundant cytoplasm which stained a homogeneous bluish red with hematoxylin and eosin, with an eccentrically placed nucleus with cartwheel formation of the chromatin material, a paranuclear vacuole in some cells, and a small centrally placed nucleolus. However, many plasma cells were not typical. Binucleated cells were frequent. Other cells, while conforming to the general structure of plasma cells, were not mature. They were larger than the adult form. The nucleus was likewise larger, with an increase in the nuclear-cytoplasmic ratio. The chromatin material was arranged in strands with irregular condensations around the periphery. Two nucleoli were common. These cells were considered immature plasma cells or plasmablasts.

Autopsy Report

Autopsy was performed 8 hours after death. Only the pertinent findings are included in this report. The margins of the upper one-third of the surgical incision were not completely approximated. This opening communicated with a small abscess cavity, 3 cm in diameter, in

the abdominal wall. No unusual findings were present at the site of resection.

An embolus was present at the bifurcation of the pulmonary arteries. It completely occluded the left branch, and partially occluded the right. There were several depressed, blue, firm areas in both lower lobes, which were considered to be due to atelectasis. The source of this embolus was not determined.

The axillary, thoracic, abdominal, and retroperitoneal lymph nodes were found to be enlarged, discrete, and soft. The largest measured 2 cm. in length and 1 cm. in diameter. Especially large nodes were found in the celiac and superior mesenteric groups, and around the head of the pancreas and in the gastrohepatic ligament. These nodes were in close approximation to the operative site. The adenopathy involved to some extent all periaortic and iliac groups and the nodes along the ascending colon. The thoracic nodes were enlarged, but not to a greater degree than is often found in routine autopsies.

Microscopic Features. The capillaries throughout the entire body were dilated and filled with erythrocytes. Albuminous fluid was present in the myocardium and in the pulmonary alveoli. Small focal areas of organizing pneumonia, large macrophages containing pigment, and a small healed infarct also were present in the lungs. There was a chronic inflammatory reaction with foreign body giant cells and organized thrombi in the small vessels at the operative site in the head of the pancreas. It was impossible to evaluate the plasma cells quantitatively in the various groups of nodes by any precise method. Since plasma cells are frequently found in nodes without any specific lesions, we used the axillary nodes as a norm, as they did not seem to have an abnormal number. The various sections of these nodes either contained no plasma cells or but a few in the stroma. This was interpreted as 1 plus. On this rough quantitative basis the nodes around the ascending colon were graded 2 plus, and the periaortic, celiac, and mesenteric nodes as 3 plus. The perigastric nodes removed surgically were 4 plus. The mesenteric nodes had plasma cells invading the capsule and extending into the perinodal fat. The mesenteric, celiac, and right axillary nodes were moderately scarred and edematous.

Anatomic Diagnoses. Plasmacytoma of stomach with lymphadenopathy of regional lymph nodes with infiltration of plasma cells; pulmonary embolus with complete occlusion of left and partial occlusion of right pulmonary artery; hyperemia of viscera; pulmonary edema and edema of myocardium; granulating surgical wound of abdominal wall; postoperative abdominal adhesions; small focal areas of organizing pneumonia; small pulmonary infarct.

Discussion

The plasma cell apparently is a definite cytologic entity. Its origin and function are still the subject of much discussion. Michels,[3] in 1931, reviewed the theories of the morphogenesis, function, and developmental capacity of the plasma cell. In his review he gave the following concepts as to its origin: (1) from connective tissue cells, (2) emigrated lymphocytes, (3) monocytes or pre-existent tissue lymphocytes, (4) immature blood cells. Lowenhaupt,[4] recently, in studying cases of multiple myeloma, concluded that the plasma cells arise from tissue histiocytes of the spleen and lymph nodes. Plasma cells are frequently present in normal lymphatic tissue according to Maximow and Bloom.[5] In view of these theories as to the origin of the plasma cell, it seems reasonable to assume that a plasmacytoma could arise in the stomach.

Plasma cells are usually present to some degree in chronic pyogenic and granulomatous lesions. They may also form specific tumors, arising primarily in the bone marrow or viscera.[1,6] Some consider that this cell may characterize a leukemic state.[7-10] In our case the histologic picture did not resemble any granulomatous process with which we are familiar. No fungi or acid-fast bacilli were found. The Kahn test on the peripheral blood was negative. We concluded, therefore, that this was a primary plasmacytoma of the stomach. Dr. Shields Warren and Dr. C. A. Hellwig reviewed the slides and concurred in the diagnosis.[11,12]

The next problem was whether this tumor was benign or malignant. The patient gave a vague clinical history of gastric symptoms for 7 years, suggesting a benign lesion. The duration, however, of an extramedullary plasmacytoma is not a criterion for either its recurrence after removal or the presence of metastases. These tumors may have a duration of 10 or more years before metastasizing. There is evidence which leads us to conclude that the lesion was malignant. The immaturity and atypical appearance of the plasma cells, and their apparent invasive qualities are in keeping with a malignant process. However, Hellwig [1] pointed out that "the microscopic appearance does not play such a dominating rôle in predicting the clinical course of a given lesion. . . . . the localization and the gross appearance seem to be more reliable criteria than the histologic structure."

The lymph nodes around the stomach, removed at the time of resection, likewise bore out the assumption that the gastric lesion was malignant. The presence of large numbers of plasma cells in the peripheral sinuses and in the stroma is readily explained by spread from the primary lesion to the regional lymph nodes. The presence,

likewise, of plasma cells in the capsule and perinodal fat gave added weight to the conclusion that this is probably an invasive lesion. Since we can find no other case reports of lesions in this location, and since the patient died 14 days postoperatively, the possibility of recurrence could not be evaluated as a criterion of malignancy.

It is interesting to note that, as far as we are able to determine, this plasmacytoma was limited to the stomach and regional lymph nodes. We found no increase in number or abnormal types of plasma cells in distant nodes, bone marrow, or peripheral blood. Many writers [6-10] have called attention to the occurrence of coexisting plasmacytomas of the marrow or viscera, and a plasma cell leukemia. In spite of the 7-year history and the size of the primary lesion, no generalization had taken place. It is interesting to speculate, but impossible to prove, whether, had widespread involvement taken place, it would have been the result of metastasis or of multicentric origin of neoplastic plasma cells.

Summary

A case of plasmacytoma of the stomach is presented. No similar case could be found in the literature. Involvement of regional lymph nodes and local infiltration indicated that this neoplasm was malignant at the time of operation.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

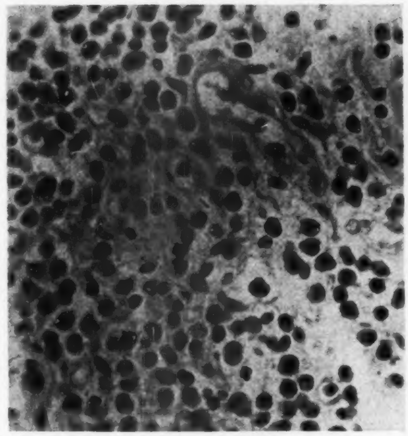
PLATE 41

FIG. 1. Lymph node along the greater curvature of the stomach, showing plasma cells in dilated peripheral sinusoids and infiltrating into the perinodal fat. Hematoxylin and eosin stain. × 100.

FIG. 2. High-power magnification of the same node as depicted in Figure 1, showing typical plasma cells. Hematoxylin and eosin stain. × 450.

FIG. 3. Pylorus, showing part of the ulcerated plasmacytoma. The cross section is through the base of the ulcer which is about two times as thick as the uninvolved stomach wall.

FIG. 4. The wall of the ulcer, showing the cellular infiltration between the muscle bundles. These cells are almost entirely plasma cells. Hematoxylin and eosin stain. × 100.



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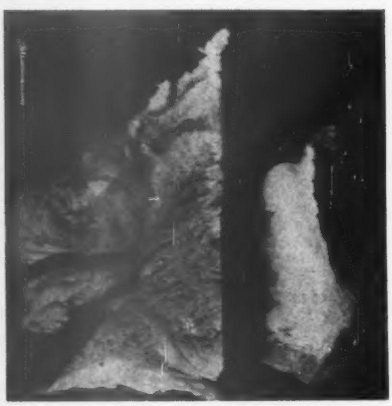

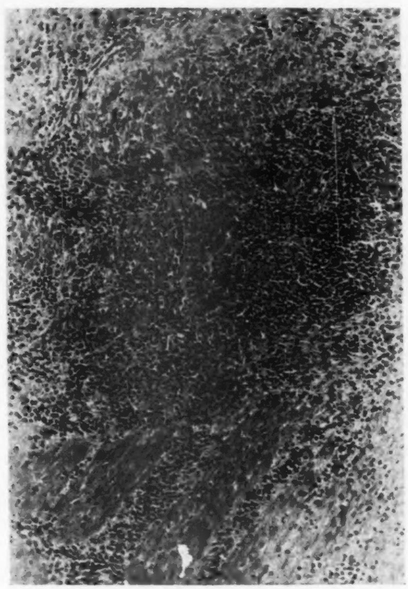

4

THE FATE OF CARCINOMA EMBOLI IN THE LUNG *

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During the routine examination of histologic sections taken from lungs of patients dying from carcinoma of various organs, outspoken thickening of the intima of smaller arteries and veins was often noticed. This was found in lungs which disclosed metastatic tumors as well as in those in which no metastases were encountered. These changes were more severe and were much more frequently encountered in patients with carcinoma than in patients of similar or older ages without carcinoma. Soon it was noted that there was a relationship between such thickened blood vessels and the presence of remnants of tumor emboli. The purpose of this investigation was to study the fate of tumor emboli in the lungs and their relation to the seeming sclerosis of the smaller blood vessels of the lung.

This study is based on the histologic examination of the lungs of 12 patients dying from carcinoma of various sources. In 5 instances the primary carcinoma was in the colon, in 2 instances in the stomach, ovaries, and cervix uteri, and once in the skin. The lungs, grossly, showed no evidence of metastasis. The ages of the patients varied from 36 to 60 years. Only those lungs were studied which histologically disclosed tumor emboli but no frank metastasis. Numerous blocks were taken from such lungs and stained with iron hematoxylin and eosin, and with orcein for elastic fibers. Serial sections were taken from several blocks.

It has been known for many years that tumor emboli may occur in the lungs in the absence of metastasis. As early as 1903, Schmidt [1] found tumor emboli in the pulmonary arterioles in 15 instances, in only 5 of which tumor cells were encountered exclusively within the vessels. Evidence of degeneration of these tumor emboli was described. Since Schmidt's publication, similar observations have been recorded from time to time. Iwasaki,[2] who studied tumor emboli histologically, concluded that tumor emboli are often made innocuous by the process of organization. Takahashi [3] noted the gradual disappearance of tumor cells in the blood vessels of the lungs of mice which had been injected intravenously with tumor cells.

Willis [4] stated that "tumour embolism is not metastasis" and that "many neoplastic emboli perish or remain sterile in their new sites of arrest." He also gave a number of pertinent references. Warren and

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Gates [5] studied the fate of intravenously injected tumor cells. They stated that, on the basis of their experimental studies, there is no doubt that tumor emboli may become organized, may disappear or remain latent over long periods without establishing true metastases. They left open the question whether this is the result of death of some of the cells, which initiates thrombus formation, or of mechanical factors, or of the resistance of the host.

In the following, a short description is given of changes within the walls of vessels which harbor tumor emboli.

Tumor emboli were found in the smaller and smallest branches of the pulmonary artery, in capillaries, and within the small pulmonary veins. In some instances the tumor cells were clumped, occupying only a relatively small portion of the lumen. Adjacent to these tumor cells were fibrin and many red blood corpuscles. In other sections tumor emboli filled the entire lumen on cross section. In some fields in which the vessel was only partially filled with tumor cells, hyaline thrombi were encountered close to the cancer cells and at a distance from them. These hyaline thrombi often were rather recent, and evidence of incipient organization was either lacking or very early. Here and there such hyaline emboli were completely or partially surrounded by newly formed lining endothelial cells. Often, tumor emboli and small hyaline thrombi were encountered side by side. In such instances it was occasionally noted also that the tumor emboli were separated from the remainder of the lumen of the vessel by one layer of lining endothelial cells. The cells constituting the tumor emboli varied, depending upon the morphologic characteristics of the primary tumor. Most commonly they consisted of individual epithelial cells, not arranged in any particular fashion even in those instances in which the primary tumors proved to be adenocarcinomas. Often the tumor cells showed oval, spindle-shaped, or round nuclei which were densely stained. Anachromasia and anaplasia were constant findings. It might be noteworthy to emphasize that the spindle-shaped tumor cells were found principally at the periphery of the tumor emboli, the nuclei in their centers being distinctly vesicular and more round.

In some sections it was noted that the tumor cells were obviously compressed by the hyaline thrombus which apparently grew by apposition of new thrombotic material. In obviously older instances, some of the tumor cells, particularly those located adjacent to the thrombi, seemingly were thinned out, had become atrophic, and finally were only indistinctly outlined. Gradually the hyaline thrombus seemed to extend into the region of the atrophic tumor cells which eventually were replaced by the thrombus. Often, masses of tumor cells were

divided into smaller accumulations and were separated from one another by thrombi. The impression was gained that the thrombus had burrowed between the clumps of tumor cells. It is interesting to note that also in these seemingly atrophic tumor cell masses, occasional atypical mitotic figures could still be recognized. Often, also, clumps of obviously compressed tumor cells were found completely encircled by a hyaline thrombus. Coincident with the growth of the thrombus and resulting organization, such clumps of tumor cells were sometimes surrounded by young connective tissue cells. Since sometimes the hyaline thrombus was at least partially surrounded by a layer of lining endothelial cells continuous with that of the vessel, cancer cells could often be noted in the small openings where the original thrombus had approximated the opposite wall. Thus the impression was gained that the thrombus was canalized, the individual channels being partially filled with cancer cells. In some of the fields adjacent to those that showed tumor emboli, hyaline thrombi in various stages of organization were often recognized completely occluding the lumina. Only rarely a few tumor cells were still recognized somewhere within the thrombus.

Remarkably often, small branches of the pulmonary arteries were encountered containing tumor cells intermixed with thrombi attached to the intima of only a relatively small portion of the blood vessel. Into such mural thrombi fibroblastic cells often were seen extending from the wall and gradually replacing the thrombus. Eventually such a thrombus was completely replaced by hyalinized connective tissue and covered by lining endothelial cells. Thus, the impression was often gained of an arteriosclerotic plaque rather than of an organized thrombus. Such plaques were found very often in the various sections taken from the lung. Occasionally within the same vessel an early organizing mural thrombus was noted upon which was superimposed a more recent tumor embolus.

Discussion

Outstanding changes were encountered within the vessels which contained tumor emboli. The histologic study indicates that these emboli, when they fail to leave the capillaries and produce metastases, cause the formation of hyaline thrombi. These thrombi either surround the tumor emboli or are formed adjacent to them when tumor cells become adherent to the vessel wall. The hyaline thrombi seemingly cause atrophy of the adjacent tumor cells, and eventually autolysis, the tumor cells being gradually replaced by the growing hyaline thrombus. The thrombus becomes organized, but often tumor cells still are found in various portions of the lumen surrounded by the organized or organizing thrombus. Thus, occasionally, canalized thrombi

are seen with clumps of tumor cells within the channels. In instances in which only mural thrombi are found, the eventual organization causes localized intimal thickening which very closely resembles pulmonary arteriosclerosis. Such localized thickening of the smaller branches of the pulmonary arteries in patients who have a primary carcinoma seems to indicate that at some time previously pulmonary tumor emboli had been present.

From the foregoing, it seems clear that it is not the tumor emboli which become "organized," but that the hyaline thrombi which very likely had been caused by the tumor emboli gradually become organized. As these thrombi grow and become organized, the tumor cells gradually disappear and are replaced by the organizing thrombus.

Willis [4] raised the question whether blood or blood clot inhibits neoplastic growth. After citing some experimental work in regard to the fate of intravenously injected tumor cells within the pulmonary blood vessels, he recommended caution before attributing intravascular degeneration and death of such introduced tumor cells to any specific anti-cancerous or inhibitory quality residing in blood plasma or blood clot. Also, Warren and Gates [5] concluded that blood has no toxic effect on tumor cells.

As was pointed out before, tumor emboli and hyalinized thrombi were encountered in both arteries and veins. Thus it seems that neither presence nor lack of oxygen plays any rôle in destroying the tumor cells. From the histologic picture it is possible that it is the mechanical injury brought about by the thrombus with consequent compression of the tumor cells which is detrimental to the cancer cells. However, it is equally clear that this cannot be the only explanation since in other lungs which were the seat of metastases, organizing thrombi and tumor emboli were simultaneously encountered in the smaller blood vessels.

The tumor cells constituting the emboli are definitely viable. They stain well and even details of nuclear structures are often clearly visible. Mitotic figures are often observed. Also, that in serial sections tumor cells were found in the smallest arteries, capillaries, and veins may speak for the fact that these cells have eventually grown into the veins. Emboli consisting of nonviable tumor cells are bound to lodge in the smallest branches of the pulmonary artery and capillaries, and to remain there. Yet, as was brought out previously, the walls of the vessels were not invaded by tumor cells in these cases.

The localized and diffuse thickening of the intima of the smaller arteries is noteworthy. As a matter of fact, it is this type of vessel change which was noted first and which stimulated a detailed examination of the lungs in these instances. The intimal thickenings are

end-stages of completely organized thrombi. Although transitions are often encountered from hyaline thrombi with tumor cells to organizing thrombi with tumor cells, in such end-stages cancer cells were never seen. It is clear that diffuse thickening of the intima, the result of organized mural thrombi, closely resembles and, as a matter of fact, often cannot be distinguished from pulmonary arteriosclerosis. This is found so frequently that it seems justified to suspect cancer emboli in the lungs of patients with cancer when histologic sections disclose unexplained arteriosclerosis of the pulmonary arterial branches.

Summary

Twelve lungs in which carcinoma emboli were encountered on routine histologic examination were studied. These emboli were found in the smaller and smallest branches of the pulmonary artery, in capillaries, and in small pulmonary veins. A small sheath of fibrin and hyaline thrombi almost invariably were encountered adjacent to the tumor cells. The thrombi seemingly caused atrophy of adjacent tumor cells which were gradually replaced by the growing thrombi. Eventually these thrombi became organized, but clumps of tumor cells were still recognizable either within the new channels or embedded within the growing connective tissue. Thus, not the tumor emboli but the hyaline thrombus became organized and caused the disappearance of the tumor cells. When only mural thrombi were encountered, the eventual organization caused localized intimal thickening which very closely resembled pulmonary arteriosclerosis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 42

FIG. 1. Tumor cells and fibrin intermingled in a small pulmonary artery. Iron-hematoxylin and eosin preparation. × 180.

FIG. 2. Carcinoma cells and fibrin in pulmonary vein. Iron-hematoxylin and eosin preparation. × 230.

FIG. 3. Of note are the tumor cells, hyaline thrombus, and organizing thrombus. Hematoxylin and eosin preparation. × 180.

FIG. 4. Hyaline thrombus with very early organization and tumor cells in a pulmonary vein. Iron-hematoxylin and eosin preparation. × 90.

FIG. 5. Organizing thrombus with superimposed carcinoma embolus. Iron-hematoxylin and eosin preparation. × 180.

FIG. 6. Small pulmonary artery with hyaline thrombus in the center and tumor emboli at both sides of the hyaline thrombus. Iron-hematoxylin and eosin preparation. × 90.

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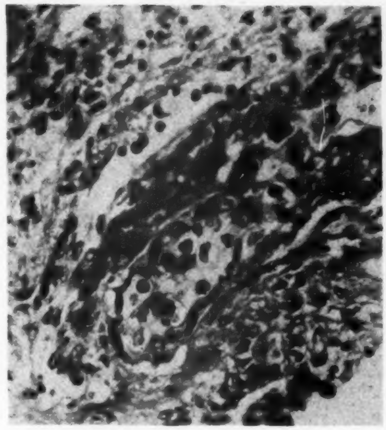

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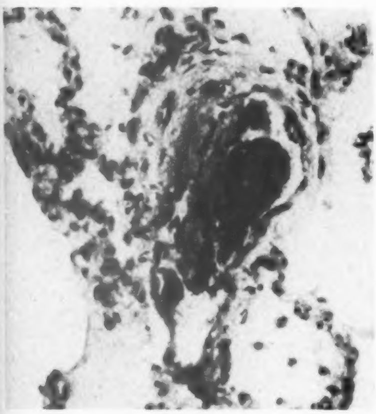

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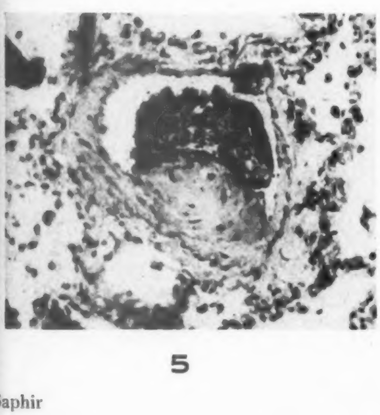

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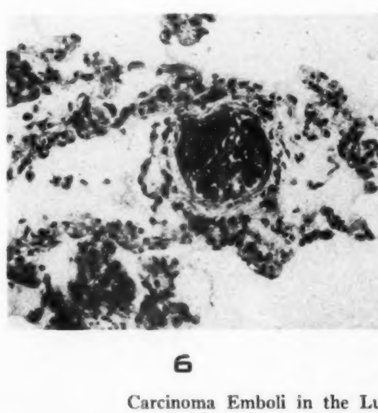

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Carcinoma Emboli in the Lung

PLATE 43

FIG. 7. Crganizing thrombus with superimposed carcinoma embolus. Of note are the endothelial cells lining the thrombus. Hematoxylin and eosin preparation. × 90.

FIG. 8. Hyaline thrombus with remnants of tumor cells. Hematoxylin and eosin preparation. × 90.

FIG. 9. Organizing thrombus with superimposed hyaline thrombus and carcinoma embolus between. Iron-hematoxylin and eosin preparation. × 180.

FIG. 10. Transformation of organizing thrombus into a knob-like intimal thickening. Iron-hematoxylin and eosin preparation. × 180.

FIG. 11. Thick-walled small pulmonary artery resembling arteriosclerosis. Hematoxylin and eosin preparation. × 180.

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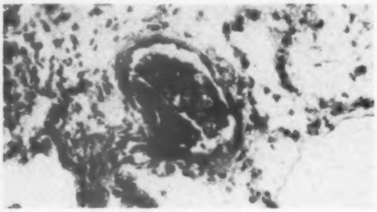

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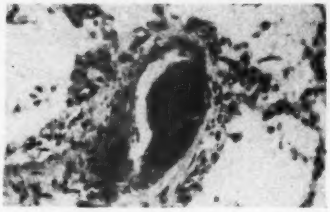

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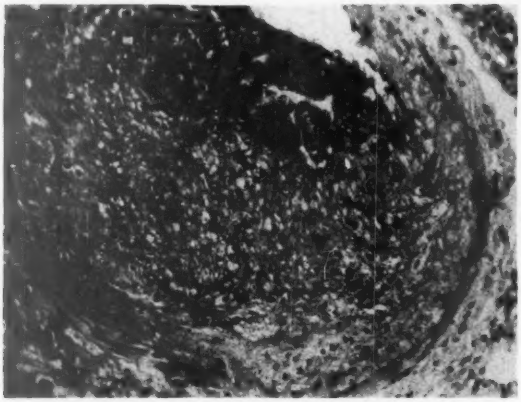

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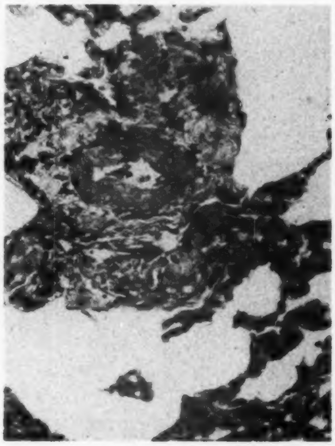

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THE PERMEABILITY OF THE RENAL GLOMERULI OF SEVERAL MAMMALIAN SPECIES TO LABELLED PROTEINS *

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While studying the origin of colloid droplets in urodeles, it was found that intravenous injection of proteins, labelled with diazotized dyes, was followed by the appearance of tiny granules, colored by these dyes, in the lining cells of the convoluted tubules of both the "closed" and "open" nephrons.[1] Because of this unanticipated finding, a systematic study was made to investigate the permeability of the renal glomerular filter of several mammalian species to labelled proteins.

MATERIALS AND METHODS

The various proteins to be used were coupled with the disodium salt of 2-naphtol-3 : 6 disulfonic acid (R salt) according to procedures described by Kabat and Heidelberger.[2] Nitrogen determinations were made with the micro-Kjeldahl method in duplicate. Three times re-crystallized egg albumin was prepared according to Heidelberger.[3] Purified solutions of serum albumin and serum globulin were prepared in the usual manner.

The preparations used in the experiments were as follows:

Neopeptone (Difco Laboratories, Detroit, Michigan)-R salt
Egg albumin (thrice re-crystallized)-R salt
Serum albumin (cat, dog, mouse)-R salt
Serum globulin (cat, dog, mouse)-R salt
Diazotized R salt alone, 0.12 per cent.

The different dilutions of these protein-dye compounds are given in the reports of the respective experiments.

PROCEDURES

White mice, white rats, albino guinea-pigs, albino rabbits, and mongrel dogs were injected intravenously with varying amounts of the above-mentioned protein-R salt preparations and were allowed to live for periods of time ranging from 30 minutes to 28 days after the injection. During this time, they were fed on the regular stock diets used for these various species.

After autopsy, the organs were fixed immediately in Zenker's fluid and in a 4 per cent solution of formaldehyde. The paraffin sections were examined either unstained but cleared, or stained with hematoxy-

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lin only, or stained with hematoxylin and light green in order to provide a color contrast. The protein-R salt compounds present in the tissues remained unaltered for years and could easily be identified, even in minute amounts, by their intense bright red color.

The Distribution of Protein-R Salt Compounds in the Organs after Intravenous Injection

Mouse Series 1

Groups of 5 mice, each weighing 30 gm., were injected with 0.5 cc. of protein-R salt preparations and the animals were sacrificed 30 minutes, 1 hour, 2 hours, 3½ hours, and 24 hours after a single injection.

(a) Peptone-R salt, 0.2 mg. of N per cc. (21.7 mg. of protein per kg. of body weight). The findings in the organs were as follows: The Kupffer cells showed a faint reddish tint in animals killed 1 hour after the injection; definite red granules were present in these cells 3½ to 24 hours after the injection, and similar granules were found in a few reticulo-endothelial cells of the spleen. The urine of all animals had a definite reddish tint. Sections of the kidneys showed no trace of red matter.

A repetition of the experiment using a concentration of 0.3 mg. of N per cc. (27 mg. of protein per kg. of body weight) gave similar results. Peptone preparations proved to be toxic to mice so that injections had to be given slowly and in small portions.

(b) Egg albumin-R salt, 1.1 mg. of N per cc. (114.5 mg. of protein per kg. of body weight). The Kupffer cells and some of the reticulo-endothelial cells in the spleen and in various other viscera showed granules and small clumps of bright red matter 30 minutes after the injection. The lining cells of the proximal portion of the renal proximal convoluted tubules, including some of the lining cells of the spaces of Bowman, contained tiny bright red granules situated within the cytoplasm (see Figs. 1 and 2). These granules became more definite and abundant the longer the animals lived after the injection, and they appeared to be present in all of the lining cells of the proximal convoluted tubules. They were never observed in any other portion of the renal tubular system.

(c) Serum albumin (cat)-R salt, 1 mg. of N per cc. (104 mg. of protein per kg. of body weight). The results of these experiments were similar to those observed in (b), except that granules in the lining cells of the convoluted tubules were first observed in animals sacrificed 3½ hours after the injection. They were more numerous and definite after 24 hours.

(d) Serum globulin (cat)-R salt, 2 mg. of N per cc. (208 mg.

of protein per kg. of body weight). A few red granules were present in the Kupffer cells only 30 minutes after the injection; 1 hour after the injection similar granules were seen also in the reticulo-endothelial cells of various viscera and tiny red dots made their appearance in the lining cells of the proximal convoluted tubules of the kidneys. These were more numerous and definite in mice which had been allowed to live a longer period of time after the injection.

Summary of Results of Mouse Series 1. Intravenous injection of protein-R salt compounds was followed by the appearance of labelled materials in the reticulo-endothelial cells of the viscera and within the lining cells of the proximal convoluted tubules of the kidneys where they appeared from ½ to 3½ hours after the injection.

Mouse Series 2

Groups of 8 mice each were injected intravenously on 2 successive days with 0.5 cc. of various protein-R salt compounds and pairs of animals of each group were sacrificed 2, 4, 13, and 28 days after the first injection.

(a) Egg albumin-R salt, 1.1 mg. of N per cc. (229 mg. of protein per kg. of body weight). In sections from animals killed 2 days after the injection, all of the lining cells of the proximal convoluted tubules contained abundant cytoplasmic red granules. The Kupffer cells, reticulo-endothelial cells, and interstitial cells of the viscera were laden with clumps and granules of red matter. Similar changes were present in the sections from mice 4 days after the administration of protein-R salt compounds. Thirteen days after the injection, only occasional clumps of granules were left in the lining cells of the convoluted tubules while masses of red matter were present in the Kupffer cells and reticulo-endothelial cells. In animals which lived 28 days after the injection, scarce small granules remained in the lining cells of the tubules while interstitial cells, Kupffer cells, and reticulo-endothelial cells contained ample red material. There was sloughing and degeneration of lining cells of the convoluted tubules so that detached cells, containing red granules, could often be seen within the lumina.

(b) Serum albumin (dog)-R salt, 0.72 mg. of N per cc. (150 mg. of protein per kg. of body weight). The findings were similar to those in (a) except that practically no granules were found in the lining cells of the renal tubules 13 and 28 days after the injection.

(c) Serum globulin (dog)-R salt, 1.17 mg. of N per cc. (244 mg. of protein per kg. of body weight). The findings were similar to those described above. Thirteen days after the injection, only a few of the

lining cells of the convoluted tubules showed groups of granules, which were rather coarse. Most of the interstitial cells exhibited phagocytized red matter and the reticulo-endothelial cells contained masses of red material. Twenty-eight days after the injection no more granules could be found in the lining cells of the renal tubules.

Summary of Results of Mouse Series 2. The number of granules within the lining cells of the renal convoluted tubules decreased steadily the longer the animals were allowed to live after the injection of protein-R salt compounds. There was desquamation of these lining cells so that only scarce red dots remained 28 days after the injection of egg albumin-R salt and serum albumin-R salt; none were seen in mice 28 days after the injection of serum globulin-R salt. The Kupffer cells, reticulo-endothelial cells, and interstitial cells of the viscera retained the material very well.

Mouse Series 3

Two groups of 6 mice each were injected intravenously one to five times on successive days with 0.3 cc. of homologous mouse serum protein preparations coupled with R salt. Two animals of each group were sacrificed 24 hours after the first injection and one animal of each group was killed 24 hours after each following injection. In addition, 2 mice were injected intravenously with diazotized R salt alone and were sacrificed 24 hours afterwards.

(a) Serum Albumin (Mouse)-R Salt, 0.5 mg. of N per cc.

(1) Twenty-four hours after the administration of 31.3 mg. of protein-R salt per kg. of body weight, some of the Kupffer cells showed a faint reddish tint and capillaries of the lungs contained reddish casts. No other changes were recognized.

(2) After intravenous administration of 63 mg. of serum albumin-R salt per kg. of body weight, abundant bright red granules were visible in the lining cells of the proximal convoluted tubules in addition to those present in the reticulo-endothelial cells and interstitial cells of the viscera.

(3) However, 24 hours after the injection of 68 mg. of serum albumin-R salt per kg. of body weight, the results were similar to those described in (a) (1). No granules were seen in the renal tubules.

(4) The results seen after the injection of 94, 125, and 156 mg. of serum albumin-R salt per kg. of body weight were similar to those described under (a) (2). The number of granules present in the lining cells of the tubules was roughly proportional to the amount of material administered (Figs. 1 and 2).

(b) Serum Globulin (Mouse)-R Salt, 0.56 mg. of N per cc.

(1) After the injection of 35.4 mg. of protein-R salt per kg. of body weight, only the Kupffer cells showed a faint reddish tint. No granules were seen in the kidneys.

(2) Injection of 50 mg. of serum globulin-R salt per kg. of body weight was followed by the appearance of a few faintly reddish granules in the lining cells of the renal proximal convoluted tubules; the Kupffer cells contained faintly stained reddish masses.

(3) After injection of 71 mg. of protein-R salt per kg. of body weight, the granules in the kidneys were definite but scarce, while those in the reticulo-endothelial cells were numerous.

(4) After intravenous administration of 106, 142.2, and 174 mg. of homologous serum globulin per kg. of body weight, abundant granules were seen in the tubular lining cells as well as in the reticulo-endothelial cells and interstitial cells of various viscera.

(c) Diazotized R Salt Alone, 0.12 per cent (12 mg. of R Salt per kg. of Body Weight)

Very few tiny red granules were present in the lining cells of the proximal convoluted tubules while numerous granules were apparent in the reticulo-endothelial cells and in some of the interstitial cells of the viscera.

Summary of Results of Mouse Series 3. Intravenous injections of protein preparations from homologous species coupled with R salt were followed by the appearance of phagocytized materials in the reticulo-endothelial cells of the viscera as well as in the lining cells of the renal proximal convoluted tubules. The number of granules depended on the amount of material administered. Injections of diazotized R salt alone produced similar results.

Rat Series

Three groups of 2 rats each, weighing, on the average, 150 gm., were injected intravenously with 3 cc. of protein-R salt compounds and the animals were sacrificed 24 hours later.

(a) Egg albumin-R salt, 1.69 mg. of N per cc. (211.3 mg. of protein per kg. of body weight). Tiny bright red granules were present in the lining cells of the proximal convoluted renal tubules, and the reticulo-endothelial cells of the viscera contained phagocytized red matter.

(b) Serum albumin (dog)-R salt, 1.83 mg. of N per cc. (229 mg. of protein per kg. of body weight). The results of these experiments were similar to those of (a).

(c) Serum-globulin (dog)-R salt, 0.95 mg. of N per cc. (119 mg.

of protein per kg. of body weight). The results of these experiments were similar to those described above, but occasional pale red granules were seen in a few lining cells of the proximal convoluted tubules.

Summary of Results of Rat Series. After intravenous injection of protein-R salt compounds into rats, phagocytized red matter was seen in the reticulo-endothelial cells of the viscera and tiny red granules were present in the lining cells of the proximal convoluted tubules.

Guinea-Pig Series

Three groups of 2 guinea-pigs each, weighing, on the average, 250 gm., were injected intravenously with from 2.5 to 4 cc. of protein-R salt compounds and the animals were sacrificed 4 hours after the injection; 2 animals were injected with diazotized R salt alone.

(a) Egg albumin-R salt, 1.69 mg. of N per cc. (102.5 mg. of protein per kg. of body weight). Some of the Kupffer cells in the liver showed a faint red coloration. There was no trace of the labelled protein in any of the other viscera.

(b) Serum albumin (dog)-R salt, 1.83 mg. of N per cc. (183 mg. of protein per kg. of body weight). The results were similar to those of (a).

(c) Serum globulin (dog)-R salt, 0.95 mg. of N per cc. (71.3 mg. of protein per kg. of body weight). The results of these experiments were similar to those of (a).

(d) Diazotized R salt alone, 0.12 per cent (14.4 mg. of R salt per kg. of body weight). No colored particles were seen in any of the viscera. R salt alone, given intravenously, proved to be quite toxic to guinea-pigs; therefore, injections had to be given slowly and in small portions.

Summary of Results of Guinea-Pig Series. With the exception of Kupffer cells, which showed a slight reddish coloration, there was no evidence of the presence of labelled proteins in any of the viscera after intravenous injection of protein-R salt compounds.

Rabbit Series

(a) One albino rabbit, weighing about 2000 gm., was injected 16 times with 1 cc. each of an egg albumin-R salt preparation containing 0.8 mg. of N per cc. A total amount of 40 mg. of protein per kg. of body weight was given and the animal was sacrificed 1 month after the first injection and 5 days after the last administration. In microscopic sections of the viscera, red granules were present in the reticulo-endothelial cells of the viscera but not elsewhere.

(b) One albino rabbit, weighing approximately 2000 gm., was

injected 16 times with 1 cc. each of a solution of diazotized R salt alone. A total amount of 9.6 mg. of R salt per kg. of body weight was given. The result was similar to that described above.

Summary of Results of Rabbit Series. There was no evidence of the presence of labelled materials in the lining cells of the renal tubules after intravenous injection of egg albumin-R salt or of diazotized R salt alone. The reticulo-endothelial cells of the viscera, however, contained phagocytized red particles.

Dog Series 1

(a) A healthy dog, weighing 10.2 kg., was injected intravenously with 65 cc. of a solution of egg albumin-R salt, containing 1.69 mg. of N per cc. A total amount of 67.3 mg. of protein per kg. of body weight was given and the animal was sacrificed 24 hours afterwards. Microscopic sections showed tiny red granules in the lining cells of the renal proximal convoluted tubules and in the reticulo-endothelial cells of the viscera.

(b) A healthy dog, weighing 12.8 kg., was injected intravenously with 120 cc. of diazotized R salt alone. A total of 11.3 mg. of R salt per kg. of body weight was given and the animal was sacrificed 24 hours later. Sections of the organs showed granular red matter in the reticulo-endothelial cells of the viscera but not elsewhere.

Summary of Dog Series 1. After intravenous injection of egg albumin-R salt, colored particles appeared in the reticulo-endothelial cells of the viscera and in the lining cells of the proximal convoluted tubules. Although red granules were present in the reticulo-endothelial cells after intravenous injection of diazotized R salt alone, the renal tubules showed no evidence of the presence of colored matter.

Dog Series 2

In order to study the renal excretion of R salt after damage to the kidneys, 3 dogs received 4.0, 6.0, and 8.0 mg., respectively, of a solution of uranium nitrate subcutaneously [4] before intravenous injections of diazotized R salt. After the applications of uranium nitrate the urine contained ample albumin.

(a) A healthy dog, weighing 11 kg., was injected subcutaneously with a solution of uranium nitrate, a total amount of 4 mg. of uranium nitrate per kg. of body weight was given. Fifty cc. of a 0.12 per cent solution of diazotized R salt were given intravenously 43 hours after the administration of uranium nitrate and, similarly, 100 cc. of R salt were injected 53 hours later. A total amount of 16.4 mg. of R salt per kg. of body weight was injected, and the animal was sacrificed 4 hours

after the last, and 54 hours after the first, injection of the R salt. At autopsy, the urine showed a faint reddish tint. Microscopic sections of the organs disclosed granular red matter in the reticulo-endothelial cells of the viscera. There was extensive necrosis of the lining cells of the convoluted tubules and within these cells were seen faintly red-stained masses of irregular sizes, but no well formed granules.

(b) A healthy dog, weighing 9.5 kg., was given 6 mg. of uranium nitrate per kg. of body weight subcutaneously. Three days afterwards 100 cc. of diazotized R salt were injected intravenously and 150 cc. were given again the following day. A total amount of 31.6 mg. of R salt per kg. of body weight was administered. The animal died 15 minutes after the last injection. At autopsy, the liver was large and dark red; the gallbladder was distended by abundant bile; the urinary bladder contained urine showing a faint red color. Microscopic sections showed changes similar to those described under (a). The lumina of the convoluted tubules were distended by protein casts exhibiting a faint reddish hue.

(c) A healthy dog, weighing 10.5 kg., was injected with 8 mg. of uranium nitrate subcutaneously, and 100 cc. of diazotized R salt were given 3 days later and again the following day. A total amount of 22.8 mg. of R salt was administered and the animal was sacrificed 5 hours after the last injection. Microscopic studies of the viscera showed results similar to those described above.

Summary of Results of Dog Series 2. After damage to the kidneys by uranium nitrate followed by intravenous injection of diazotized R salt, ill defined, faintly red-stained masses were found in some of the necrotic lining cells of the renal convoluted tubules; however, no well formed granules were seen. Colored particles were present in the reticulo-endothelial cells of the viscera.

Discussion

The presence of labelled protein particles within the lining cells of the renal convoluted tubules after intravenous injection of various protein-dye compounds indicated that such substances were able to pass the glomerular filter. This conception hinges on the validity of the assumption that the diazotized dyes are inseparably coupled with the protein molecule serving as a label by which the presence of the respective protein can be recognized. This was proved chemically by Kabat and Heidelberger [2] and by Smetana and Johnson [1] in electrophoretic experiments.

To what extent the proteins are denatured by coupling them to the R salt is not known. In anaphylactic experiments with guinea-pigs,

using native proteins for sensitization, and proteins coupled with R salt for the final injection or vice versa, no differences were observed; likewise qualitative as well as quantitative precipitin reactions with either coupled antisera against native antigen or vice versa gave results identical to those with controls.[5]

The amount of protein passing through the normal glomerular filter is probably too small to be detected by ordinary laboratory methods, while even minute amounts of protein-R salt preparations can be visualized microscopically. The filtering membrane apparently does not differentiate between foreign or homologous species proteins. No studies were made with homologous serum proteins coupled with R salt; however, it is assumed that diazotized R salt injected into the blood stream combines with the serum proteins, thereby forming labelled homologous serum protein compounds. In mice, these compounds did pass through the glomerular filter, as the presence of tiny red granules within some of the lining cells of renal convoluted tubules indicates.

The passage of protein substances of relatively large molecular sizes, such as serum albumin and serum globulin, through the normal glomerular filter is rather surprising and changes to some extent the physiologic conception of the function of the filtering membrane of the renal glomerulus. However, it has been shown that even under normal condition some protein is regularly escaping into the glomerular filtrate from which it is absorbed by the lining cells of the proximal convoluted tubules.[6]

The appearance of labelled protein particles within the lining cells of the proximal convoluted tubules strongly suggests that they are absorbed by these cells from the glomerular urine. Although secretion of these substances by the lining cells has to be considered, this seems unlikely because the granules first make their appearance in the lining cells of the funnels of the convoluted tubules or even in the lining cells of the spaces of Bowman before they can be seen in the supraglomerular loops, but are never found in cells of any other portion of the renal tubular system. If they were excreted by the lining cells of the tubules, one would expect colored material in the urine to appear for some time after the injection, which is not the case. It is realized, however, that only direct observation of the nephron during an acute experiment can settle this problem definitely.

The fate of the granules within the lining cells of the tubules is linked with the length of life of the cells in which they are situated: their number diminishes, due to desquamation of lining cells, until none or very few are left. In mice this takes about 1 month after the last

administration of protein-R salt compounds. The phagocytized particles of labelled protein compounds in the Kupffer cells, reticulo-endothelial cells, and in the phagocytic interstitial cells of various viscera remain indefinitely.

The failure to demonstrate labelled protein granules within the lining cells of renal tubules in experiments with guinea-pigs and rabbits perhaps indicates species differences. However, when the amount of injected protein-dye compounds per kg. of body weight is computed, it appears likely that too little material was given to rabbits, so that the results obtained in this series are inconclusive (Table I). Similarly,

TABLE I

Tabulation of Results to Show Quantitative Level at Which Granules Appeared in the Renal Tubular Epithelium

Animals	Mg. of substrate per kg. of body weight				
	R salt	Peptone	Egg-albumin	Serum-albumin	Serum-globulin
Mouse series	$\underline{12.0}$	21.7	$\underline{114.5}$	31.3	35.4
Mouse series		27.0	$\underline{229.0}$	$\underline{63.0}$	$\underline{50.0}$
Mouse series				68.0	$\underline{70.8}$
Mouse series				$\underline{93.8^{*}}$	$\underline{106.0^{*}}$
Rat series			$\underline{211.3}$	$\underline{229.0}$	$\underline{119.0}$
Guinea-pig series	14.4		102.5	183.0	71.3
Rabbit series	9.6		40.0		
Dog series	11.3		$\underline{67.3}$		

Underscored figures indicate granules in renal tubules; figures not underscored indicate no granules in renal tubules.

* Amounts larger than these always gave positive results.

the results obtained in mice with peptone-R salt preparations are inconclusive; due to the toxicity of this preparation, larger amounts were not tolerated.

In general, it can be stated that the greater the amount of protein-R salt given, the more extensive were the deposits found in the lining cells of the renal convoluted tubules as well as in the reticulo-endothelial cells of the various viscera. A minimal amount of about 60 mg. of protein per kg. of body weight has to be administered before granules appear in the lining cells of the kidney tubules (Table I).

After intravenous injection of diazotized R salt alone into dogs following damage of the kidneys due to uranium nitrate, small amounts of this dye passed through the glomerular filter and faintly stained masses of red substance were seen in some of the necrotic lining cells of the convoluted tubules. This is in contrast to the brilliantly stained, well defined granules which appear in the tubular cells of normal animals injected with protein-R salt compounds. It is suggested that

the formation of these granules is an expression of a normally functioning cell and this has perhaps a bearing on the interpretation of colloid droplets as being protein particles stored in functioning lining cells of tubules after re-absorption from tubular lumina of protein substances which have passed through the glomerular filter.

Conclusions

1. Preparations of egg albumin, and of serum albumin and serum globulin of heterologous and homologous species, labelled by R salt, pass the glomerular filter of normal mice, rats, and dogs after intravenous injection; particles of these substances are re-absorbed by the lining cells of the proximal convoluted tubules after passage through the glomerular filter and are stored in these cells in the form of tiny granules.

2. The number of granules present in the lining cells of the tubules is roughly proportional to the amounts of protein-dye compounds administered.

3. The particles of protein-dye compound remain in the lining cells of the tubules until these cells are desquamated.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

Plate 44

Fig. 1. Mouse series 3 (five intravenous injections on successive days of homologous mouse serum albumin-R salt, 0.5 mg. of N per cc., totalling 156 mg. of protein per kg. of body weight). Section of kidney, showing a glomerulus, the space of Bowman, and the funnel of the proximal convoluted tubule. The tiny black dots within the cytoplasm of the lining cells of the convoluted tubule and in some of the cells lining the space of Bowman represent brilliantly stained red granules of the serum albumin-R salt. × 1050.

Fig. 2. Section of kidney from the same animal as shown in Figure 1. The small black dots in the cytoplasm of the lining cells of the proximal convoluted tubules represent the intensely stained red granules of the serum albumin-R salt. × 1050.

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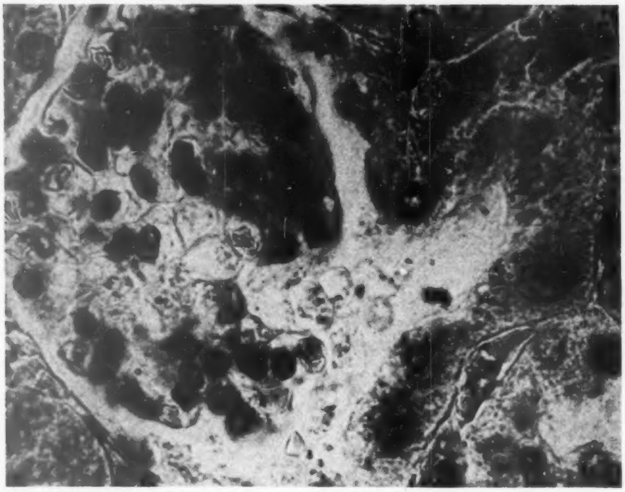

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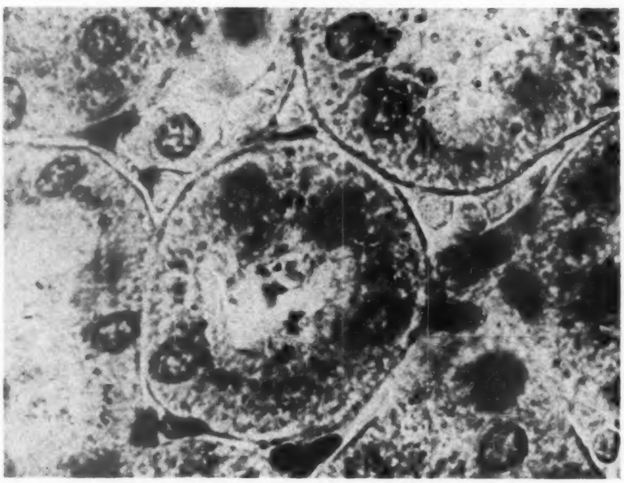

2

Smetana Permeability of Renal Glomeruli

INTRALOBULAR REGENERATION OF LIVER CELLS IN MAN *

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Regeneration of liver tissue is a well recognized phenomenon, having been described in 1833 by Cruveilhier.[1] Much experimental work has been done in an attempt to elucidate the mechanisms concerned. Most of these observations have been made in connection with regeneration which follows partial hepatectomy, and that which results from extensive chemical injury of the liver cells. Ponfick,[2] in 1889, clearly demonstrated the phenomenon of regeneration after hepatectomy, and many others have contributed similar information in more recent years (Milne,[3] Fishback,[4] Brues, Drury, and Brues,[5] Sulkin [6]). The matter of mitotic or amitotic division of cells in the regenerating liver [7] and the actual means of enlargement of the regenerating lobule (Mann [8]) still appear to be somewhat controversial. Few factors which regulate or influence liver cell regeneration have been determined. Mann and Magath [9] demonstrated the necessity of an intact portal circulation, and Brues, Drury, and Brues found that diet exerted an important influence on regeneration.

In the study of human material one has frequent opportunity to note extensive liver cell regeneration, particularly in cirrhosis and in instances of acute and subacute necrosis of more or less extensive form produced by a variety of agents. Our attention has been drawn recently to the fact that definite evidence of regeneration of liver cells without nodule formation exists with great frequency in human livers in which neither cirrhosis nor extensive liver cell necrosis has been present. Regeneration of this type has been largely neglected in previous studies, and it is with this type that the present study is concerned.

Methods of Study

A series of 100 autopsy cases was studied without selection except that the records of a few infants were discarded so that they would not constitute too large a proportion of the group. Also, cases of cirrhosis were excluded. The clinical records and autopsy reports were consulted for significant findings. The principal study consisted of a histological investigation of the livers in which observations on the presence, degree, morphological characteristics, and distribution of the regenerative phenomena were made, and other lesions in the liver described. This report deals with an analysis of these histological findings as related to certain clinical and gross anatomical features.

* Received for publication, April 5, 1946.

Incidence

In order to determine the frequency of the aforementioned histological changes in routine autopsy cases it was necessary to establish a baseline of normal histological appearance of the liver. Even in cases of accidental death the liver sometimes showed slight to moderate regeneration. More or less arbitrarily, we have decided to classify as essentially within normal limits those livers in which there was either no evidence whatever of regeneration or only very rare areas of modified, regenerating cells. Thirty-seven per cent, according to these criteria, were normal livers as far as regeneration was concerned, while 30 per cent showed 1 plus; 21 per cent, 2 plus; 6 per cent, 3 plus; and 6 per cent, 4 plus regeneration.

Table I

Distribution as to Age of Livers Showing Evidence of Regeneration

Age	Total no.	No. of cases without regeneration	No. of cases with regeneration			
			+*	++	+++	++++
(years)						
Below 1	8	7	1	0	0	0
1–10	2	2	0	0	0	0
11–20	7	6	0	0	1	0
21–30	5	2	2	1	0	0
31–50	26	14	5	5	1	1
51–70	35	5	17	9	2	2
71–90	17	1	5	6	2	3

* An arbitrary quantitative differentiation of the regeneration, based on the experience in this series of cases.

Age

It was found that evidence of regeneration appears much more commonly in adults than in infants and children. However, definite regeneration, never to a marked degree, was occasionally noted in the young. The distribution according to age is given in Table I.

Morphological Characteristics

Cells revealing evidence of regeneration were modified in appearance with respect to both nucleus and cytoplasm. The most characteristic change consisted of enlargement of the nuclei. These often were found to be one and one-half times the normal diameter and sometimes as much as three times the normal. The nuclei showed somewhat greater variation in shape than is normal, often being oval, but usually they were rounded, and sometimes quite irregular. These enlarged nuclei were characterized, further, by an increased amount of chromatin. Nucleoli were more prominent than usual, increased in number to five or six, and almost always larger than the normal liver cell

nucleoli. Many of these hyperchromatic and enlarged nuclei were double, that is, the liver cells were binucleate.

The cytoplasm was increased in amount, although some exceptions to this were noted. The cytoplasm was usually more opaque, and rather paler than normally. Fat droplets and bilirubin pigment were noted often in the cytoplasm of regenerating cells. Occasionally, areas of regeneration were characterized by a syncytium-like arrangement, and the cells did not form discrete cords. Usually, however, large liver cords were formed by the cells, or the regenerating cells occupied positions in otherwise normal-appearing cords.

Concerning the distribution of the areas of regeneration, considerable variation existed. Usually they were focal, either in the center or periphery of the lobule. In every case the lobular architecture of the liver was retained. The designation "intralobular regeneration" would seem advisable in order to distinguish this process from the nodular regeneration of cirrhotic and post-necrotic livers. In those instances in which local retrogressive lesions were present in the liver, the regenerating cells were located in the area of the lesion or immediately around it. In several instances, especially when no other histological abnormalities were noted in the liver, the process of regeneration was diffuse throughout the lobule. Binucleate liver cells were more numerous in areas of regeneration, but only in one case were mitotic figures observed, and in this instance they were very numerous.

The hypertrophied nuclei of regenerated liver cells were frequently found to contain rounded, pale, eosinophilic inclusions similar to those noted by Andrew, Brown, and Johnson.[10] They, also, often contained fat droplets and bile pigment granules. The amount and appearance of the fat and bile coincided with that noted in the normal liver cells in the remainder of the section.

Association with Other Pathological Changes

The majority of cases of focal regeneration were found in patients who had chronic passive hyperemia of the liver. There were some cases, however, of long-standing hyperemia of the liver with atrophy of cells in the lobular center in which no regeneration was present. In those in which regeneration occurred, it was present largely in the zone of liver cells just peripheral to the atrophic or necrotic zone. Of 25 cases of chronic passive hyperemia with central atrophy or necrosis, 19 showed evidence of regeneration, the majority of which were 1 plus (Fig. 1). Of 8 cases of acute hyperemia, only one showed regeneration; this case was graded 1 plus.

There were 18 cases in which some degree of fatty metamorphosis

of the liver cells was encountered. In 5 there was no evidence of regeneration while in the remaining 13 cases 1 to 2 plus regeneration was found (Fig. 2). If any correlation existed between the degrees of fatty infiltration and of regeneration, it was an inverse correlation. The regeneration was found to involve cells containing fat droplets about as often as those which were free from fat. Binucleate cells were also noted among the fat-containing cells. Regeneration was accentuated in the cells around the foci of fatty infiltration.

In one group of cases there was marked emaciation. Usually, in this group, the livers were smaller than normal. There were 17 such cases, and of these there was no regeneration of the liver cells in 4. In 13 there was well marked regeneration. The liver cells which were not involved in the regenerative process were somewhat smaller than is normal and the cytoplasm darker. In these cases no other pathological changes were noted in the liver.

In the absence of other factors, infectious diseases did not appear to be associated with regeneration of liver cells.

In the 2 cases of eclampsia, regeneration was marked. There were 2 cases of central necrosis due to toxic agents, and in these regeneration was very marked.

There were 26 cases in which no factors were known, or hepatic lesions recognized, which might have a bearing on the incidence of regeneration. Of these, 14 cases showed no evidence of regeneration. These constituted a large portion of the normal livers of this series. In the remaining 12 some degree of regeneration was present. Interestingly enough, most of the cases of 4 plus regeneration fell in this category (Fig. 3). In some of these cases the regenerative activity was essentially diffuse in distribution, producing considerable enlargement of the lobules. The architecture of the lobules, however, was never disturbed.

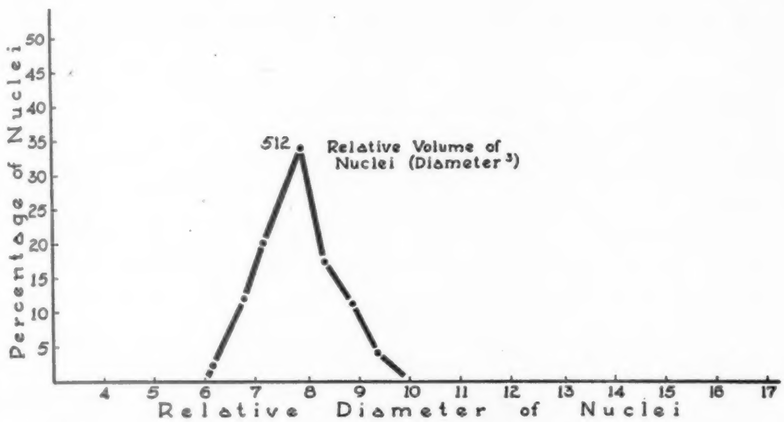
Size of Nuclei of Regenerating Cells

Beams and King [11] and Sulkin [6] have recently described the nuclear changes in the regenerative cells of the liver of the partially hepatectomized rat. They noted marked increase in the size of nuclei and suggested that such hypertrophied nuclei were due to polyploidy. This conclusion was reached in view of the fact that peaks of incidence of liver cells of various sizes occurred in such a way as to indicate the presence of nuclei of two, four, eight, etc. times the volume of normal nuclei.

With this possibility in mind for human liver undergoing regenera-

tion, we have plotted the size of liver cell nuclei in normal and in regenerating livers. This was accomplished by the projection of cells at a standard magnification and drawing the nuclei directly on the image. From 100 to 200 nuclei, without selection, were drawn in each case studied and the drawings then measured.

The nuclei of normal human liver fall in a narrow range and are characterized by a single peak as to size (Text-Fig. 1). In those instances in which regeneration has occurred, the curve of nuclear size extends over a wide range, shifting markedly to larger sizes, with several small peaks of the nuclei of large sizes occurring in each case (Text-Fig. 2). It appears that these peaks fall at such intervals that, by calculation, the volume of the nuclei is roughly twice or four times

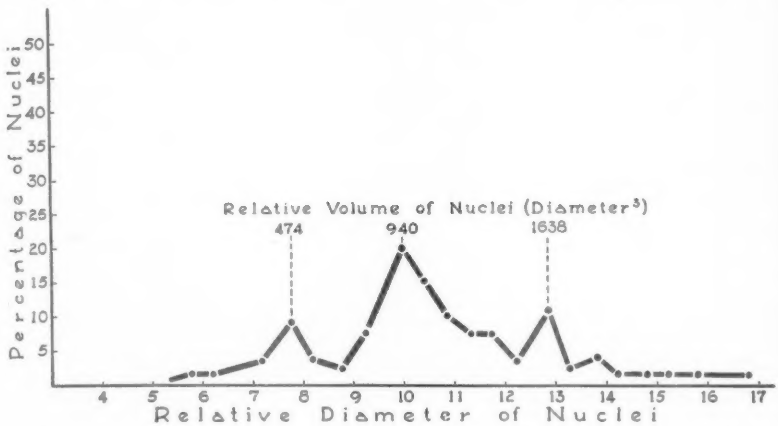


Text-Figure 1. Curve showing distribution of nuclear size in a normal liver.

the normal size. According to this criterion then, one might assume that polyploidy occurs in regeneration of human liver cells. According to Beams and King,[11] this process involves the development of binucleate cells, fusion of chromosomes of the two nuclei at a single mitotic spindle, and subsequent division with the formation of two cells, the nucleus of each having twice the normal chromosome component, that is, being tetraploid. Repeated mitosis with failure of cytoplasmic division, according to Beams and King, occurs in the rat, with the formation of octaploid, 16-ploid, etc. nuclei. Our findings would suggest a similar occurrence in man except for the almost complete absence of mitotic figures in the human liver undergoing regeneration. This matter will be considered further under the next topic.

Binucleate Cells

Cells containing double nuclei are well known to occur in the normal liver. Sulkin [6] found them to be increased in the rat in livers which were undergoing regeneration following partial hepatectomy. In the human liver without evidence of regeneration we have found binucleate cells to constitute about 4 per cent of the cells of the periphery of the lobule and 7 per cent at the center. In regenerating livers the percentage of binucleate cells averaged 11 and 14 per cent, respectively, at the periphery and center of the lobules. Binucleate cells were more numerous in the area where regeneration was active. In addition to binucleate cells, multinucleated liver cells containing as many as five or six nuclei were noted occasionally in the areas of regeneration. Most of the nuclei of binucleate cells were of about normal size, *i.e.*, diploid, but occasionally they were tetraploid. Mitotic figures were never en-



Text-Figure 2. Curve showing distribution of nuclear size in marked intralobular regeneration of the liver. 76-year-old male with no other lesions in the liver.

countered in binucleate cells, but every stage of what appeared to be amitotic division was found. This consisted of slight elongation, constriction at the middle of the elongated nucleus, and almost complete separation of the two segments of the dividing nucleus. The cytoplasm of cells with nuclear division proceeding in this manner was often eosinophilic and the same feature was noted in some cells in which the two nuclei were separate. In one case in which there was very active regeneration, mitotic figures were present in large numbers (Fig. 4). They occurred in areas where there was other evidence of regeneration, and in no instance were double nuclei encountered in the cells under-

going mitosis. If the mechanism of polyploidy occurs in the binucleate cells, however, single mitotic figures would be expected even if the cells were previously binucleate.

Regeneration without Local Lesions in the Liver

A remarkable phenomenon noted in several cases was that of marked diffuse regeneration without evidence of liver cell injury. Grossly, there were no changes in these livers except in a few in which faintly demarcated, small nodules could be made out on the cut surface; some were described as having a lighter brown, somewhat yellowish color. Microscopically, such livers showed on careful study that the faint nodular outlines were simply enlarged, rounded lobules with essentially normal architecture. The cells were very large and the nuclei prominently exhibited the changes listed as characteristic of regeneration. Occasionally, larger poorly outlined nodular areas seemed to compress adjacent liver tissue.

Analysis of the cases in which this appearance was noted did not reveal the cause of the regeneration. It seems probable that some form of mild active liver injury was occurring in these cases, perhaps in the nature of a dietary injury, which exaggerated the normal process of necrobiosis of liver cells and hence increased the rate of their new formation, producing the picture of regeneration.

Discussion

Andrew, Brown, and Johnson [10] described hypertrophied and hyperchromatic nuclei in the livers of senile rats and men. As far as we can determine, the appearance of cells here described is similar to that described by them. It is possible that regeneration in senile animals occurs more commonly than in younger animals. MacNider [12] noted that regeneration in the young animal occurs readily with the formation of cells morphologically similar to the normal cells while regeneration in the older animal is characterized by the formation of larger cells which are more resistant to injury. Our observations are in accord with those of MacNider in this respect. Hypertrophied and hyperchromatic nuclei, although occurring occasionally in the young, are rare. It might be assumed that regeneration of liver cells in the older age group is characterized by polyploidy occurring in many of the dividing cells, and to a greater degree than is encountered in younger persons. Another factor to be taken into consideration in explaining the difference in incidence of regeneration in the young and old age groups is that those factors which seem to be associated with the occurrence of regeneration; namely, chronic passive hyperemia, fatty

infiltration, and emaciation, are seen far more commonly in older persons.

The mechanism of regeneration of liver cells has been the source of considerable controversy. Milne [8] found no evidence of participation of bile ducts. He explained the presence of small, solid bile duct "buds" as due to the fact that the connecting ducts between canaliculi and interlobular ducts are rendered more prominent through collapse. He considered newly formed cells to arise from other liver cells. Our findings are in complete accord. In our cases of focal regeneration there seems to be no question of the exclusive origin of regenerated cells from other liver cells. It appears that regeneration may proceed in any portion of the lobule. In instances of central liver cell injury, the regeneration develops in this region and in other cases the location of the injury determines the position of the regeneration. In view of the greater number of binucleate cells around the central vein as compared to the periphery, one may assume regeneration to occur more readily around the center, as Sulkin [6] indicated in his study of regeneration in the rat.

Regeneration in the liver might conceivably proceed through mitosis or amitosis. With the exception of one case, we noted no evidence of mitosis whatsoever. However, in this one instance it is certain that regeneration was largely through this mechanism. Beams and King,[11] and Sulkin [6] considered mitotic division to be the sole mechanism of regeneration in the rat. We found abundant morphological evidence suggesting amitotic division in practically every case of regeneration in man and are led to believe that this is by far the most important means of regeneration in the human liver, but that regeneration by mitotic division also may occur.

In these instances of regeneration it was noted that the lobular architecture of the liver was well preserved. This would seem to indicate that there is little likelihood of this process passing into cirrhosis. Destruction of total lobules or more extensive intralobular destruction with subsequent regeneration, on the other hand, characteristically may lead to portal cirrhosis.

Many local lesions of the liver which lead to atrophy, degeneration, or necrosis are followed by an appropriate regenerative response. In some cases, however, when regeneration was absent, there was evidence of destruction and injury of the liver cells. Such cases were marked by the rapid occurrence of death after the onset of the disease. This was found to occur particularly with acute hyperemia and degeneration of the liver cells. The greater the duration of injury, the more marked was the regeneration.

In several cases of extreme fatty infiltration, no evidence of regeneration was found. Some of these patients, however, died as a result of hepatic failure, and since the process was of many days' duration, it is believed that such altered liver cells may be incapable of regeneration.

Summary

As opposed to nodular regeneration in the human liver following extensive necrosis and in cirrhosis, focal or diffuse intralobular hyperplasia is a very common finding in autopsy material. In a series of 100 cases this type of regeneration was found in 63 per cent. The prominent characteristics of this process consist of hypertrophy and hyperchromatism of nuclei, increased numbers of binucleate liver cells, and increased amount of cytoplasm.

The lesions in the liver commonly found to be associated with regeneration of this type were chronic passive hyperemia, fatty infiltration, and senile and malnutritional atrophy. Regeneration was found also in marked degree in connection with eclampsia and in a group of elderly patients without other apparent causes. In this latter group some of the most advanced degrees of intralobular regeneration were encountered.

Although mitotic figures were not observed in these regenerating areas of liver tissue, there was evidence of polyploidy, a phenomenon of karyokinesis occurring in binucleate cells. Thus, the increase of nuclear volume in the regenerating cells as compared to normal cell nuclei approached two to one or four to one.

It appears that in spite of the occurrence of advanced degrees of intralobular liver regeneration in many of these cases, no evidence of impaired function of the liver presents itself. Nor is there any suggestion that the lesion may proceed to the nodular type of regeneration characteristic of cirrhosis. On the other hand, the phenomenon is considered to be an expression of continuous and active cell replacement in the liver, which is speeded up in instances in which there is excessive intralobular cell loss, and which is characterized morphologically by the occurrence of polyploid nuclei and increased numbers of binucleate cells.

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DESCRIPTION OF PLATES

Plate 45

Fig. 1. Liver showing chronic passive hyperemia with moderate intralobular regeneration. Numerous binucleate cells with dark, eosinophilic cytoplasm. Hypertrophied, hyperchromatic nuclei. × 600.

Fig. 2. Liver with fatty infiltration, showing slight regeneration. × 600.

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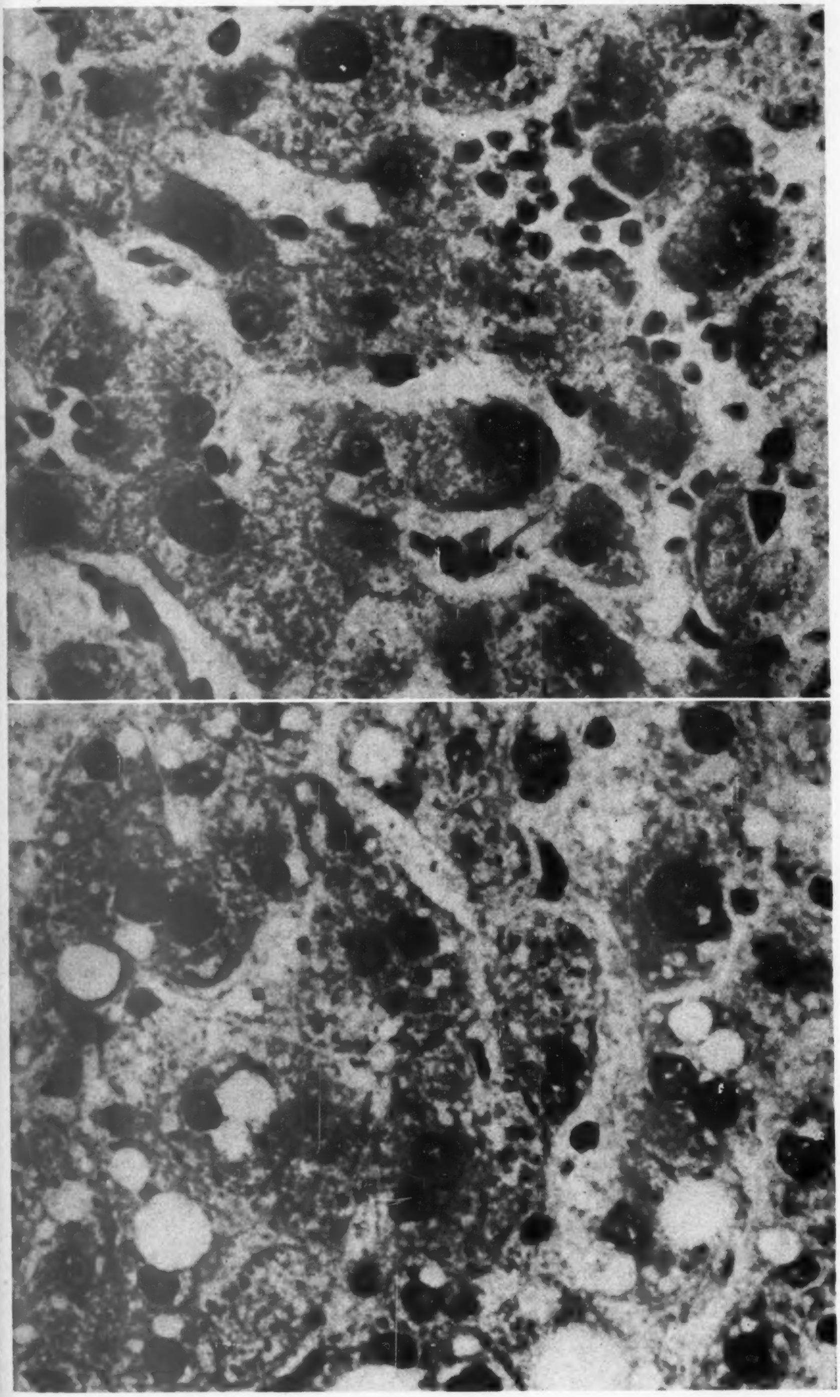

Plate 46

Fig. 3. Marked intralobular regeneration in a man, 76 years old. There were no other lesions in the liver. × 600.

Fig. 4. Moderate regeneration of the liver with numerous mitotic figures. One mitotic figure has an atypical, bifid spindle. × 600.

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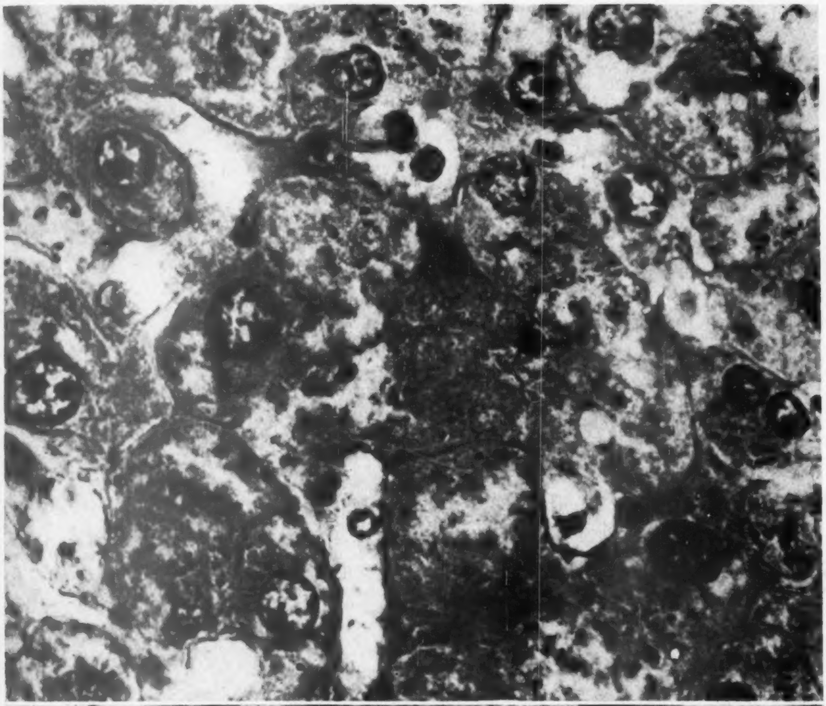

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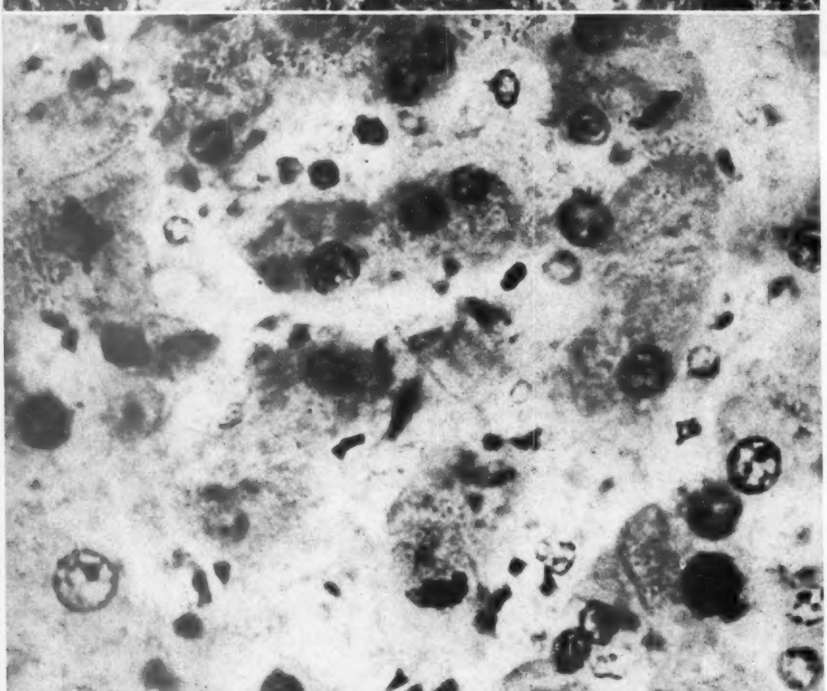

GIANT CELL TUMOR OF BONE

A Critical Survey *

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The giant cell tumor of bone with its curious cytologic structure has stimulated the imagination of a great number of writers. Indeed, a few hours with the literature on the lesion impresses one with the volume and variety of the conflicting ideas which have been expressed as to its nature. Theoretical observations concerning the lesion are hardly justifiable unless the author intends to simplify its definition. Such is the purpose of this discussion. Because this tumor is comprised of cells of two morphologically different types, it is set apart from most other neoplasms. I believe it is because these cells are found in other lesions, the nature and behavior of which are so different, that the enigma of the giant cell tumor has defied a universally satisfactory explanation.

The lesion has been described as a reparative reaction to injury. It has been explained as a granulomatous response to inflammation. Thus some writers have denied its inclusion among the tumors. On the other hand, among those who believe it to be of neoplastic character there is scarcely more accord. Geschickter and Copeland,[1] for example, stated that the tumor is a neoplastic proliferation of embryonic osteoclasts, while numerous other writers, among whom Jaffe, Lichtenstein, and Portis [2] are prominent, believe the tumor is essentially a fibrous growth characterized by the presence of the less important giant cells. The giant cells have been described as wandering phagocytes, as endothelial derivatives, and as megakaryocytes, but to the best of my knowledge they have always been considered an intrinsic part of a specific pathologic entity.

Not until comparatively recent years have three important bone diseases been described and set apart. These lesions are hyperparathyroidism and its distinction from the solitary bone cyst, fibrous dysplasia, and eosinophilic granuloma. Because all of these conditions may present a cytologic structure of giant cells in a fibrous matrix, they were almost certainly confused with giant cell tumors in the earlier literature.

In the light of accumulated knowledge there seem to be certain indisputable features of the usual concept of the giant cell tumor of bone. First of all the growth is composed of spindle cells which are of mesenchymal origin, among which are found multinucleated giant

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cells and, much less frequently, large cells with a vacuolated cytoplasm described as foam cells containing lipid. It occurs predominantly in the third and fourth decades of life in the epiphyses and adjoining metaphyses of long bones. It is a neoplasm since it produces new tissue, often recurs, and may metastasize.[3] The relationship of this particular tumor to the other lesions characterized by a fibrous ground substance and giant cells will be discussed. This relationship becomes more understandable if one can accept certain theories concerning the genesis of the cells which constitute these lesions.

True adult fibrous connective tissue is probably only one of several specialized products which arise from a common ancestral tissue represented in postnatal life by mesenchyme. Other derivatives of the same stem material are bone, cartilage, fat, and the reticulum. Mesenchyme persists throughout life and serves, among other functions, as a mother substance for the regeneration of new tissue or the replacement of destroyed tissues which lack the potentiality of regeneration. From it fibroblasts arise and produce supportive and scar tissue. Chondroblasts and osteoblasts also stem from this source to produce their respective adult analogues. From reticulum probably come the myeloid elements of bone marrow itself.[4] Cells of phagocytic function commonly termed monocytes, phagocytes, or histiocytes are now generally assumed to arise from reticulum.[5] Indeed, the fibroblast itself is endowed with phagocytic potentialities. The histologist admits the difficulty and sometimes impossibility of differentiating the macrophage and the young fibroblast on a morphologic basis.

The multinucleated giant cells found in a variety of conditions and locations throughout the body offer interesting material for speculation.[6] The large cell which is produced in the reaction engendered by a foreign body is thought to be the product of a fusion of several macrophages which are commonly found in the region. The Langhans' cell of tuberculous lesions may have the same origin, but it is found among epithelioid cells which are probably nothing more than macrophages or fibroblasts arranged in a characteristic pattern. It is interesting to note that neither of these cells displays much phagocytic activity as demonstrated by ingested particles. It is as though they had satisfied their phagocytic purpose in swallowing each other or fusing to form one cell body with only the separate nuclei to testify to their original identity.

The osteoclast, another multinucleated giant cell, has been the vortex of a maelstrom of contention among pathologists writing on the pathology of bone. There has never been any considerable unanimity

concerning its origin, but its function now quite clearly seems to be different or at least qualified from that originally ascribed to it by von Kölliker.[7] A ponderous academic discussion concerned with whether the osteoclast destroys healthy bone makes up a large share of the literature on this cell. Pommer [8] contended hotly in the affirmative, but numerous more recent workers are agreed that while the osteoclast may play an unimportant rôle in bone resorption, its chief function is that of an ordinary phagocyte.[9] It is found in tissues which produce fibroblasts and among those cells which in themselves are phagocytic. It seems logical to assume that osteoclasts represent a fusion, the fusion itself being of a phagocytic nature, of these cells.

The giant cell in the tumor of that name is the one which concerns us here. If it occurred only in giant cell tumors, we should admit the justification of the contention that the osteoclasts may become endowed with neoplastic properties to produce the tumor which writers with this belief term osteoclastoma, though even so it explains only one type of cell in the tumor and the prognostically unimportant one at that. But the giant cells of giant cell tumor, or cells morphologically indistinguishable, are found in a number of lesions with as many different causes. It is true that the giant cells of some giant cell tumors have certain features which set them apart from the osteoclast and the latter may often show characteristics which enable one to distinguish it from the giant cells of the brown tumor of hyperparathyroidism and unicameral cyst, but close scrutiny of a number of these lesions convinces me that the cells are often identical. If we begin with the premise that osteoclasts and the giant cells of giant cell tumor of bone, giant cell tumor of tendons, osteogenic sarcoma, fibrous dysplasia, hyperparathyroidism, unicameral cyst, osteoid osteoma, eosinophilic granuloma and allied reticulo-endothelioses, nonosteogenic fibroma, and ossifying fibroma are generically and functionally related, then the nature and differential diagnosis of benign giant cell tumor of bone become considerably simplified because without its giant cells the giant cell tumor of bone becomes simply a benign or malignant tumor of fibrous connective tissue—a fibroma, or a fibrosarcoma. I believe that such is the case and that all or most of the peculiarities of the giant cell tumor of bone can be logically explained on this premise.

In order better to comprehend the cytologic makeup of giant cell tumor and the conditions which produce similar microscopic pictures, a clinical, roentgenographic, and microscopic study of 115 primary lesions of bone was undertaken. The selected lesions were all noninflammatory, with the possible exception of the reticulo-endothelioses.

These were included because of the difficulty which may arise in differentiating eosinophilic granuloma and giant cell tumor of bone. The incidence of the various lesions considered is shown below:

Giant cell tumor of bone	4
Giant cell tumor of tendon	12
Fibrous dysplasia	7
Hyperparathyroidism (von Recklinghausen's disease)	1
Unicameral cyst	8
Paget's disease	7
Osteoid osteoma	3
Osteogenic sarcoma	9
Enchondroma	4
Osteochondroma	17
Chondrosarcoma	12
Periosteal fibrosarcoma	6
Osteogenesis imperfecta	3
Ewing's tumor	9
Plasma cell myeloma	4
Myeloid myeloma	3
Reticulo-endothelioses	5
Primary epithelial cell tumor of bone (adamantinoma, tibia)	1
Total	115

Giant Cell Tumors of Bone

The Giant Cells. The osteoclast was once believed to be the sole agent which brought about bone resolution. We now know that demineralization of bone is a chemical reaction depending upon a number of factors, chief of which are probably the blood supply and the reaction of the fluids which bathe the part affected. After demineralization the soft fibrous matrix disintegrates because it is not living fibrous tissue but merely the collagenous product of fibrous cells. As elsewhere in the body, this tissue débris is cleared by cells the purpose of which is phagocytosis. In the brain, phagocytosis is accomplished by the gitter cells, modified microglia; in the soft tissues, it is accomplished by macrophages and microphages which are derived from the reticulum either directly or by modification of fibroblasts. In bone, this important work is carried out by cells which in the embryo apparently arise directly from the mesenchyme and in postnatal life from the reticulum.[10,11] Just as in soft tissues in which the macrophages may be formed from abnormally proliferating fibroblasts, as in the tubercle or the foreign body reaction, so in bone they may be formed from fibroblastic cells which are exposed to an abnormal growth stimulus. Osteoclasts in abnormal numbers are seldom if ever found in healthy bone; they appear only after the lacunar cells are dead and bone has begun to disintegrate. Osteoclasis is usually a normal physiologic

process: in the embryo it clears the way for normal bone growth, in postnatal life it assists in the normal growth and maintenance of bone at a much slower pace. In abnormal situations, osteoclasts are found in large numbers most often in the presence of hemorrhage. Hemorrhage and its attendant change in the pH of the fluids and perhaps the extent of the blood supply cause bone resolution. That the osteoclast, itself an adult, functioning end-product, should acquire the proliferative characteristics of neoplasia is inconceivable. Giant cell tumors, no matter how malignant, rarely if ever show giant cells undergoing mitotic division. The giant cells may be the product of fused fibroblastic tumor cells or perhaps of incompletely separated littoral cells, but there is no evidence to show that they are self-propagating. In giant cell tumors any unusual features of their structure may be accounted for by their peculiar genesis, *i.e.*, from tumor cells. Their presence is the response to bone destruction caused by the tumor cells proper.

The Fibrous Matrix. The fibrous matrix of giant cell tumors is composed of cells of fibroblastic origin no more unusual than those of other fibrous tumors. It is these cells, however, as Jaffe [2] has so rightfully insisted, that determine the behavior of the tumor. A histologic diagnosis of giant cell tumor of bone must not be made unless the cells of the fibrous matrix are neoplastic in character. It is disregard of this very point which has led to such wild confusion in the descriptions of this tumor in the literature. The reason that many people believe that giant cell tumor is merely a response to trauma is because they have mistaken reparative cells for neoplastic fibrous cells. Their preoccupation with the giant cell has led them to consider two lesions, of dissimilar nature and prognosis, to be the same because one feature —the giant cells—was the same. In a like manner the behavior of the tumor can be predicted only by a consideration of the cells of the fibrous matrix. The giant cells give little or no clue as to whether the tumor will recur or metastasize, despite the advice from numerous quarters that the number of nuclei is inversely proportional to the degree of malignancy of the tumor.

The presence of foam cells is variable but they rarely make up any considerable part of the tumor. I have seen one giant cell tumor of a rib and another of a femur, however, in which some sections showed massive areas of these cells. I believe that the giant cell itself is made up of an agglomeration of matrix cells whereas the foam cell is elaborated directly from the reticulum. The hemorrhage and invaded marrow provide considerable amounts of lipid, and since these cells are phagocytic their cytoplasm soon becomes engorged. The presence of

foam cells does not distinguish a giant cell tumor of tendon from one of bone.

I am not implying that the histologic diagnosis of giant cell tumor of bone is an easy one. The most experienced pathologists may mistake an unusual reparative fibrous hyperplasia for tumor. Until this distinction can be made, one must be guarded about a diagnosis of giant cell tumor. Because of the inaccessibility of the lesion, insufficient material is often submitted by the surgeon, or the tumor is "uncorked" by removing a piece of the overlying cortex and the cork rather than the lesion itself is sent in for cytologic study. Even when adequate tissue is submitted, the pathologist should not hesitate to study the clinical history and the roentgenograms before venturing a diagnosis.

It is unquestionably true, as Jaffe [2] has pointed out, that giant cell tumors of bone rarely occur in young adults before the age of 20 years, and that they show a predilection for the epiphyses and the adjoining metaphyses of long bones. These characteristics are hard to explain, but an explanation is no more necessary to this discussion than is one for osteogenic sarcoma, unicameral cyst, osteoid osteoma, or the fibromas, each with its own site of predilection and age incidence. One is tempted to accept the theory that neoplasms of bone are most apt to occur at the site of growth where new cells are being formed, which, in the cylindrical bones, is at the epiphyseal line. Why the site of origin of the giant cell tumor is more often in the epiphysis than in the metaphysis where other medullary fibromas seem more prone to arise, is unknown to me unless it is because the more embryonic nature of the tissue of the epiphysis is more conducive to giant cell formation than other tissues, and growth therein establishes the peculiar nature of the tumor.

If the hypothesis that giant cell tumor of bone is a fibroma or fibrosarcoma with an unusual giant cell proliferation is correct, there might be some justification for suggesting a change of the name. Any such attempt, however, would certainly be futile. Furthermore, the tumor deserves a special designation because of its cytologic peculiarity. Perhaps the terms "giant cell fibroma" and "giant cell fibrosarcoma" might be more direct and explicit.

Differential Problems

Giant Cell Tumor of Tendons. The structure of ligaments and tendons bears many striking similarities to that of bone. The tissue is composed essentially of bundles of fibrils between which are caught spindle cells as lacunar cells are caught between lamellae of bone.

Tendon tissue is a product of mesenchyme just as is bone, although morphologically it more closely resembles ordinary fibrous connective tissue. Tumors may arise from the tendon itself or from its sheath. Those from the latter are usually classed with the endotheliomas as synoviomas or angiomas. Those arising from the tendons are collagen-producing and are therefore fibromas or, rarely, fibrosarcomas. Chronic inflammatory reaction is particularly prone to result in a productive lesion which may closely simulate a neoplasm. In the true tumors there are large numbers of giant cells and often a greater number of foam cells than in the giant cell tumor of bone.[12] Essentially, the course of this tumor is comparable to that of the giant cell tumor of bone with similar matrix components. The giant cell tumors of tendons grow slowly and, because they are more accessible, removal is usually not difficult. A few are malignant, extending along the course of the tendon and metastasizing late. Histologic differentiation from giant cell tumor of bone may be impossible, but unless the tumor has destroyed a considerable amount of bone, examination of roentgenograms should complete the diagnosis.

Osteogenic Sarcoma. During the enchondral formation of bone some of the mesenchymal matrix cells line up along the columns of cartilage to become osteoblasts. As the cartilage disappears the cells lay down osteoid tissue which later becomes calcified to form primitive bone. The osteoblasts from the inner layer of periosteum, or those from the medullary portion of the epiphyseal line may undergo neoplastic proliferation. These cells attempt to form bone, sometimes fairly successfully but more often so crudely that it may be difficult to recognize. Osteoblasts are derived from mesenchyme and they may fuse to form giant cells. Sometimes these cells are indistinguishable from those found in giant cell tumors, and since the tumor matrix is composed of collagen-producing spindle cells it may be virtually impossible to distinguish this tumor morphologically from a malignant giant cell tumor. The distinguishing feature is osteoid formation since the stem cell of the giant cell tumor normally produces fibrous connective tissue while the osteoblast produces bone. The problem becomes more complex when trying to differentiate the more "adult" type of giant cell tumor which may produce some bone, or the "young" type of osteogenic sarcoma in which bone formation is exceedingly crude or lacking. This circumstance suggests that the two lesions may merge one into the other depending upon the state of embryonic development of the stem cell. When the lesion is medullary, violates the cartilaginous end-plate, and is found in a patient over 20 years of age, the distinction had better

be postponed until more tissue can be studied or until the course of the lesion makes diagnosis possible. The only consolation to the pathologist, if not to the patient, is that if the distinction is difficult the lesion is surely malignant.

Fibrous Dysplasia. Fibrous dysplasia, particularly the monostotic variety, has been misdiagnosed roentgenographically as giant cell tumor of bone. This mistake should not be made after cytologic study. It is true that the matrix is composed of collagen-producing spindle cells, but, although they are young cells, they do not show the criteria for new growths.[13] An even more obvious difference is the production of primitive bone in fibrous dysplasia. The cells of giant cell tumor produce almost no bone whatsoever; where unquestionably produced by the tumor cells, it is a primitive, irregular type of osteoid tissue. Giant cells are often present but almost never in the numbers seen in giant cell tumor of bone. Incidentally, fibrous dysplasia, if both the monostotic and polyostotic varieties are included,[14] is by no means a rare disease. In my series of 115 primary lesions of bone there were 7 cases, while for the same period there were only 4 unquestionable cases of giant cell tumor.

Hyperparathyroidism (von Recklinghausen's Disease of Bone). Hyperparathyroidism is quite rare. In my series there was only one unquestionable case. Histopathologically, it is perhaps the lesion most easily confused with giant cell tumor of bone. Brown tumors are often quite numerous, interspersed among areas of cystic degeneration and osteoid proliferation. They are composed of masses of giant cells in a fibrous stroma. The cells of the latter should furnish the distinguishing feature. Yet if they are quite actively proliferating, I am not sure that I would care to make the distinction with finality. Some writers, including Jaffe,[15] believe that the giant cells of the two lesions are different. In the one case of my series I am unable to confirm this distinction.

Solitary Unicameral Cyst. The solitary unicameral cyst is easily mistaken for giant cell tumor on roentgenologic evidence alone, although the epiphysis is seldom, if ever, involved. When the lesion is reasonably early, the age incidence is a helpful diagnostic feature.[16] There were 8 cases in my series, the oldest patient being 21 years of age. In the healing stage of a cyst there may be enough fibrous proliferation and giant cell formation to present a histologic picture not unlike that of giant cell tumor, but here again the giant cells are usually not numerous and the fibrous matrix lacks neoplastic characteristics.

Osteitis Deformans (Paget's Disease). The solitary focal type of

Paget's disease [17] in relatively young people might conceivably produce a roentgenogram which would simulate that of giant cell tumor. Histologically, the clear-cut mosaic pattern of the bone is not always present and there may be enough fibrous proliferation and giant cells in some areas to make a diagnosis of giant cell tumor seem reasonable. Usually the clinical history, the roentgenograms, and the cytologic structure, if considered together, will result in a correct diagnosis.

Osteoid Osteoma. Although there may be numerous giant cells in a fibrous matrix in the lesion which Jaffe [18] has named osteoid osteoma, the presence of calcified osteoid tissue usually makes the histologic diagnosis an easy one.

Reticulo-endotheliosis. Cases of reticulo-endotheliosis comprise a fascinating series, the complete pathology of which has not yet been established.[19] In my series there was one atypical case of Letterer-Siwe's disease, one well defined case of Hand-Schüller-Christian's disease, and 3 cases of eosinophilic granuloma. The last is the most likely to be mistaken for giant cell tumor of bone. When the eosinophilia is prominent the pathologist has little difficulty in arriving at a diagnosis, but sometimes eosinophils are few or absent [20] and giant cells may predominate in the section. When such is the case the evidence of inflammation must be relied upon to make the distinction.

Nonosteogenic Fibroma and Ossifying Fibroma. Nonosteogenic fibroma and ossifying fibroma derive their distinction as entities from their cytologic composition. In the former there are insufficient giant cells to warrant the diagnosis of giant cell tumor; in the latter the presence of considerable amounts of primitive bone serve as the distinguishing feature. One may question the advisability of complicating the classification of bone tumors by considering these tumors as separate entities.[21] If the giant cell tumor of bone were called giant cell fibroma, this objection might be overcome by considering all three lesions as variants of the same class. My experience with the fibromas is too limited to make any observation worth while.

Conclusions

I have presented the belief that the giant cells found in a number of bone lesions are identical or generically related cells produced for the purpose of phagocytosis. They should not be used for the purpose of distinguishing a giant cell tumor of bone. Fibrous matrix cells are found also in a number of bone lesions, both neoplastic and non-neoplastic. It is unfortunately true that many diagnoses of giant cell tumor of bone are based on these two criteria alone, a mistake which

makes the incidence abnormally high. This mistake is enhanced by the acceptance of roentgenologic characteristics which are common to a number of entirely different lesions.

The histologic diagnosis of giant cell tumor of bone should not be made unless the fibrous matrix cells are definitely neoplastic in character. Whether this tumor should be considered an entity apart from the other fibromas of bone is a question I am unable to answer. There seems to be some justification for this distinction in the clinical characters of the lesions. If they were called giant cell fibroma, nonosteogenic fibroma, and ossifying fibroma, the terminology would be self-explanatory and the classification would be simplified.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Figure 1 was taken from a giant cell tumor of bone. Any of the other lesions illustrated might have been misdiagnosed giant cell tumor of bone on a cytologic basis alone, if the part examined were restricted to the areas containing giant cells in a fibrous matrix. Indeed, several of the lesions were so diagnosed by reputable pathologists. The structure of the giant cells varies considerably even within the limits of the same lesion and similar variations are found in a variety of lesions of different types. All of the sections illustrated were stained with hematoxylin and eosin and photographed at a magnification of 300.

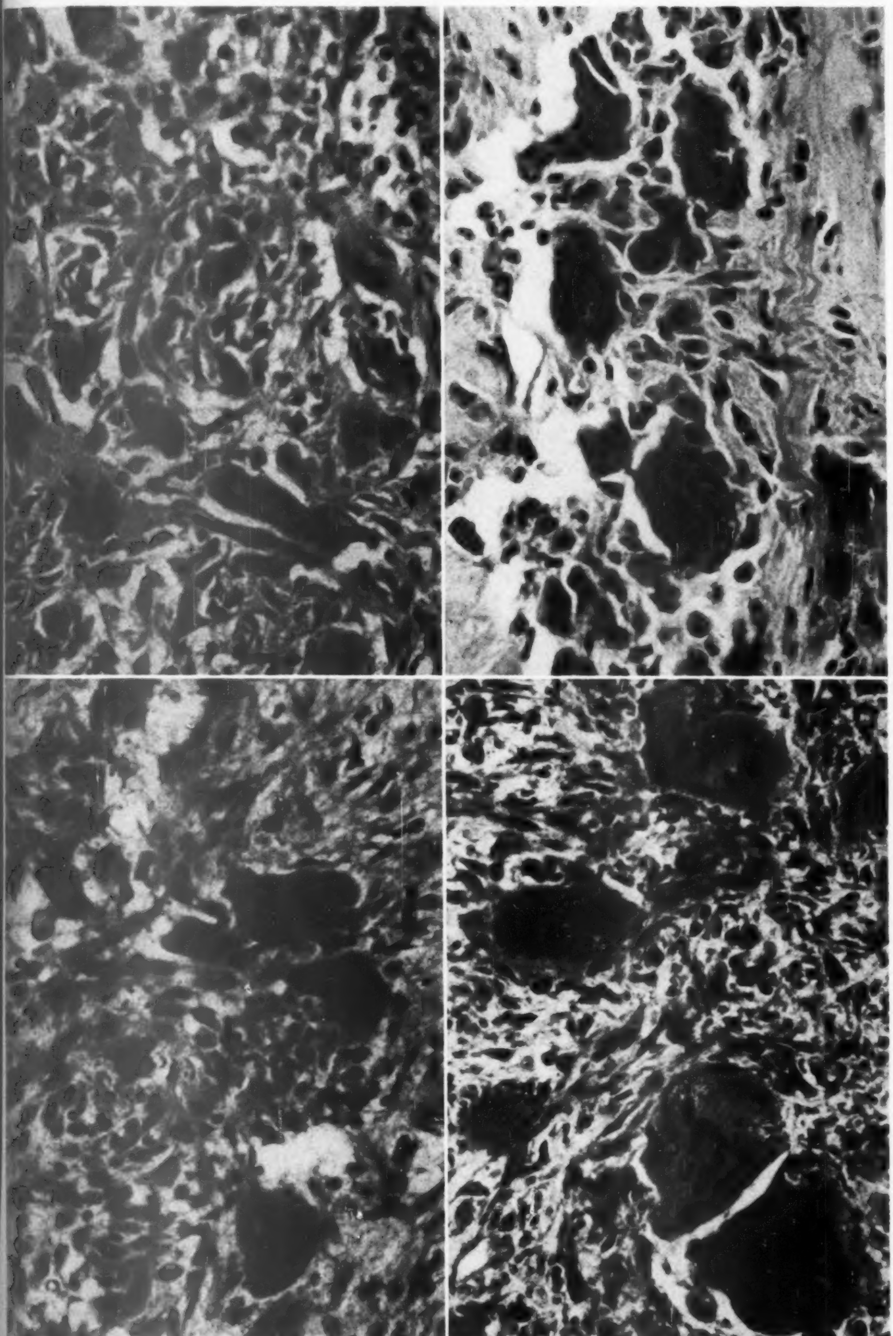
PLATE 47

FIG. 1. Male, 22 years of age. Giant cell tumor of bone: destruction of rib. Surgical resection brought about complete healing. There was no recurrence 8 years later. The giant cells varied considerably in size, shape, and number of nuclei. Some resembled those in Figure 4, an instance of hyperparathyroidism.

FIG. 2. Female, 43 years old. Giant cell tumor of tendon: tumor of tendons of palm. Surgical resection brought about complete healing. There was no recurrence 6 years later.

FIG. 3. Male, 11 years of age. Fibrous dysplasia: demineralization and deformity of the shaft of the left tibia. Treatment was by curettage and introduction of bone chips. There was no advancement of the process after 7 years.

FIG. 4. Female, 25 years old. Hyperparathyroidism: focal demineralization of femur, humerus, skull, vertebrae, and phalanges and moderate generalized demineralization, of 3 years' duration. Serum calcium was 11.9 mg. per cent. Calculi were present in both kidneys. A parathyroid adenoma, 1.5 cm. in diameter, was removed. Death occurred on the second postoperative day.



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Aegerter

Giant Cell Tumor of Bone

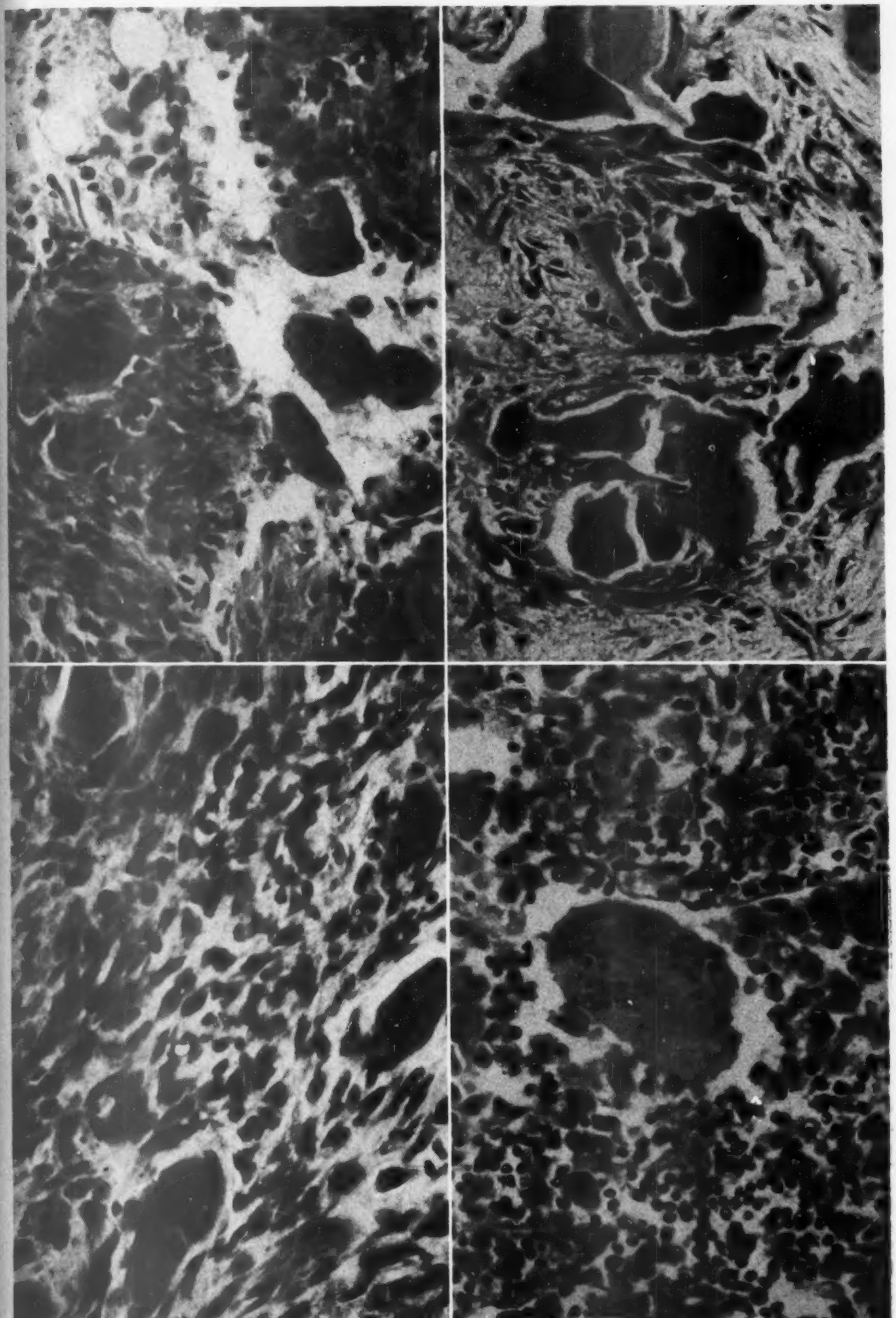
PLATE 48

FIG. 5. Female, 6 years old. Unicameral cyst: focal demineralization in the metaphysis of the left tibia. Curettage effected complete healing. There was no recurrence 7 years later.

FIG. 6. Male, 42 years of age. Paget's disease: thickening and mottled demineralization of the skull and extremities, fracture of femur and tibia. Specimen taken for biopsy revealed the typical mosaic of Paget's disease. Subsequent course was not recorded.

FIG. 7. Male, 25 years old. Osteoid osteoma: focal demineralization of the proximal radius, of 2 years' duration. The head of the radius was excised. There was no recurrence after 7 years.

FIG. 8. Female, 29 years of age. Eosinophilic granuloma: focal demineralization of the 7th and 8th ribs and the 7th thoracic vertebra. The 7th rib was resected; the other lesions regressed after 2 years.



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Aegerter Giant Cell Tumor of Bone

RESIDUAL TISSUE CHANGES IN MALE DOGS FOLLOWING CESSATION OF ORALLY ADMINISTERED STILBESTROL *

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The clinical and pathologic changes in male dogs which had received oral stilbestrol were described in a previous paper.[1] When the third dog presented in that experiment was autopsied several months after the cessation of medication, he showed certain anatomic findings which were thought to represent the residual effects of the stilbestrol. In order to investigate these organic lesions further, four other male mongrel dogs were fed stilbestrol. One succumbed early in the experiment. The other three were examined anatomically at varying lengths of time after withdrawal of the drug. The significant clinical, hematologic, and anatomic abnormalities observed in these five animals form the basis for this report.

Protocols of Experiments

Dog S-E was a long-haired, springer-spaniel mongrel about 2 years old and weighing 13.6 kg. Following a rib resection for marrow studies on January 20, 1945, he received 770 mg. of stilbestrol in 5 doses of 10 mg. and 48 doses of 15 mg. in the 60 days inclusive of January 29 through March 29, 1945. This was an average dose of 56.6 mg. per kg., or 0.94 mg. per kg. per day. On the 49th day he was inappetent, listless, and thin. By the 58th day, he had lost much weight and appeared toxic. The floor of his mouth was swollen and hemorrhagic and he began to pass bloody, semiliquid stools. He failed rapidly, lapsed into coma, and died on the 61st day, March 30, when he weighed about 9 kg. The wound of the skin for rib resection was broken down, hemorrhagic, and crusted. The hair at the operative site was not fully regenerated. The significant gross findings included severe loss of body fat; hemorrhagic right auricle; pulmonary hypostasis; small flabby spleen; widespread ecchymoses in the gastrointestinal mucosa; flabby kidneys with swollen bright yellow cortices and pale pink pyramids; fairly plentiful, semifluid, dark red costal and vertebral bone marrow; a small, soft prostate, and testes about one-third normal size.

Dog S-F was a moderately long-haired, St. Bernard-pointer mongrel, about 2 years old and weighing 15 kg. Following removal of marrow from a rib for biopsy on February 3, 1945, he received 1910 mg. of stilbestrol in one dose of 20 mg., and 126 doses of 15 mg. in 180 days inclusive of February 6 through August 4. This was an average dose of 127.3 mg. per kg., or 0.71 mg. per kg. per day. On the 77th day, when he had received 935 mg. of the drug, his appetite decreased and he began to lose weight and vigor. The stilbestrol was stopped for 21 days, during which time his general condition improved toward normal. On the 192nd day, he weighed 16.8 kg. and showed enlarged breasts, moderate swelling of the penile sheath, testes about three-fourths normal size, prostate about two-thirds normal size, general thinning of the hair, and loss of hair from the ventrum of the abdomen, the penile sheath, the perineum, and adjacent thighs. Serum agglutination for *Leptospira canicola* was positive in dilutions of 1:10 to 1:100 and for *Leptospira*

* The diethylstilbestrol used in these experiments was furnished through the courtesy of Dr. D. C. Hines of Eli Lilly and Company.

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icterohaemorrhagiae in dilutions of 1:10 to 1:1000. On the 220th day, September 13, 40 days after cessation of stilbestrol, the breasts, testes, and penile sheath were within normal limits and the hair was partly regenerated in areas from which it had been lost. He also displayed increased fullness in the ventral abdominal wall, giving the impression of a female type of body configuration, also seen in dogs S-C and S-A to be described. The significant gross findings at autopsy of dog S-F included short, pale yellow streaks at the corticomedullary junction of the kidneys and a slightly contracted prostate.

Dog S-C was a short-haired, greyhound mongrel about 2 years old and weighing 19 kg. Following removal of marrow from a rib for biopsy on December 23, 1944, he received 3520 mg. of stilbestrol in 176 doses of 20 mg. in 208 days inclusive of January 9 through August 4, 1945. This was an average dose of 185.3 mg. per kg., or 0.89 mg. per kg. per day. By the 116th day, the hair had partly regenerated at the site of the rib resection and was much lighter brown than the adjacent hair. On the 288th day, hair loss involved most of the ventral abdominal wall, the perineum, adjacent thighs, and the penile sheath. The breasts were enlarged, the prepuce swollen, and the prostate and testes shrunken. He weighed 20.5 kg. On the 332nd day, December 6, 124 days after cessation of the stilbestrol, the hair, prepuce, penile sheath, and testes were within normal limits, but the breasts were slightly enlarged and the prostate was somewhat shrunken. No other significant gross changes were observed at autopsy.

Dog S-A was a short-haired, terrier-bull mongrel, about 3 years old and weighing 11.5 kg. Following removal of marrow from a rib for biopsy on December 29, 1944, he received 1770 mg. of stilbestrol in 177 doses of 10 mg. in 208 days inclusive of January 19 through August 4, 1945. This was an average dose of 153.9 mg. per kg., or 0.74 mg. per kg. per day. By the 219th day, when he weighed 12.3 kg., the breasts were enlarged, the penile sheath was greatly swollen and hairless, the testes were shrunken, the prostate was enlarged and moderately hard, and the ventral abdominal wall and perineum were devoid of hair. On the 381st day, January 24, 1946, 173 days after cessation of stilbestrol, the hair had been regenerated and the prepuce, penile sheath, and testes were within normal limits. The breasts were slightly, and the prostate moderately, enlarged. The significant autopsy findings included a hypertrophied urinary bladder and an enlarged prostate with excavations in the dorsal parts of the lateral lobes filled with cheesy material.

Dog 52, the third dog described previously,[1] was a long-haired, water-spaniel mongrel about 1½ years old and weighing 8 kg. Following removal of marrow from a rib for biopsy on March 17, 1942, he received 1850 mg. of stilbestrol in 280 days inclusive of April 20, 1942, through January 25, 1943, and 500 mg. of the same compound subfascially on the 280th day. This was an average oral dose of 231 mg. per kg., or 0.83 mg. per kg. per day. On the 294th day, he showed enlarged breasts, greatly depressed libido, a swollen penile sheath, shrunken testes, and extensive hair loss. On the 583rd day, the clinical appearance of this dog was normal and did not appreciably change by the 791st day, May 16, 1944, 511 days after cessation of the stilbestrol, except as related to the site of implantation of methylcholanthrene as reported.[2] His final weight was 10.5 kg.

The brains of all five dogs were grossly normal.

Methods and Microscopic Observations

The tissues obtained at autopsy were fixed in Zenker's fluid, imbedded in paraffin, cut at 6 μ, and stained with hematoxylin and eosin, except that the right adrenal glands of the dogs were fixed in 4 per cent formaldehyde and sections of them were frozen with carbon dioxide gas, cut at 15 μ, and stained with sudan IV. The smears of

costal marrow were stained by the May-Grünwald-Giemsa method following preparation by the serum technic. The pituitary glands cut in sagittal section were stained by Mallory's connective tissue stain. The tissues examined microscopically included the thymus, thyroid and parathyroid glands, the heart, lungs, spleen, esophagus, stomach, intestines, liver, gallbladder, pancreas, adrenal glands, kidneys, bladder, prostate, vasa efferentia, testes, epididymides, breasts, lymph nodes, bone marrow, skin, skeletal muscle, and pituitary gland.

Adrenal Glands

In dog S-E, the adrenal cortex (Fig. 1) was moderately increased in width and had a smooth border applied evenly to the regular capsule. The shrunken cells of the zona glomerulosa had pyknotic nuclei and acidophilic cytoplasm containing few to many small granules of lipid. The nuclei of the small fascicular cells were condensed to varying degrees and the acidophilic cytoplasm contained little or no fat. Many fascicular cells, especially those along the inner edge of the fascicular zone, had eccentric pyknotic nuclei, were enlarged and rounded, and inclosed large globules of fat. An increase of the cells in the reticular zone resulted in an absolute widening of this layer and accounted for the greater thickness of the cortex. The cells of the zona reticularis resembled those in the fascicular zone, but were more flattened and contained decidedly fewer granules and globules of fat.

In dog S-F, the adrenal cortex (Fig. 2) was quite narrowed and scalloped in irregular folds beneath the capsule, which dipped down between stretches of undulated thinned cortex. The moderately shrunken glomerulosal cells showed slightly condensed nuclei, increased cytoplasmic acidophilia, and irregular decrease of fat. The fascicular layer was greatly thinned by the decrease in size and lipid content of the constituent cells, which contained nuclei condensed to varying degrees, even to pronounced pyknosis. The reticular layer was moderately thickened and the reticular cells contained little or no fat.

In dog S-C, the adrenal cortex (Fig. 3) was narrowed and peripherally scalloped. The glomerulosal cells were crowded together, showed increased cytoplasmic acidophilia, contained varying amounts of fine fat droplets, and had fairly well preserved nuclei. Especially in the outer third of the fascicular zone, the cells were smaller, severely depleted of lipid, and marked by condensed nuclei, also seen in scattered cells in the inner two-thirds. Most of the zona reticularis lacked lipid.

In dog S-A, the adrenal cortex (Fig. 4) had fairly uniform thickness and a smooth outer border. The glomerulosal cells were smaller, bunched together, and marked by fine fat granules. The cells of the

outer margin of the zona fasciculata were shrunken and depleted of lipid. The cells in the remainder of this zone were filled with fine and medium fat granules, save for a few scattered cells with condensed nuclei and cytoplasm ballooned by large fat globules. The unevenly widened zona reticularis consisted of partly flattened cells containing few fat granules.

The adrenal cortex of dog 52 was not appreciably different from that of dog S-A. The adrenal gland (Fig. 5) of a control dog had an outer cortical border, well rounded beneath the regular, smoothly applied capsule. The intact cells of the glomerulosal and fascicular layers were entirely filled with fine fat droplets. The width of the zones was evenly developed, especially that of the fascicular zone. The quite narrow reticular zone contained small numbers of partly degenerated cells swollen with large globules of lipid.

Kidneys

In the kidneys of dog S-E, the stroma, especially of the cortex, contained numerous infiltrations of lymphocytes, plasma cells, and monocytes which surrounded damaged or atrophic tubules. Several groups of atrophic convoluted tubules were imbedded in increased fibrous connective tissue infiltrated by similar inflammatory cells. With both Levaditi and Dieterle stains many spirochetes with the structure of Leptospira were present in these infiltrations, both free and within macrophages, in the form of intact organisms and disintegrated granules. Masses of spirochetes (Fig. 6) and many granules were present in the lumen and intermingled with the lining cells of several fairly well preserved convoluted tubules.

In the kidneys of dog S-F, radial, cortical, interstitial infiltrations of lymphocytes and a few pigmented macrophages surrounded atrophic tubules and glomeruli with fibrotic capsules. In both cortex and medulla irregular, radial areas of increased fibrous connective tissue were present. A few glomeruli were obliterated by fibrous connective tissue. Groups of dilated collecting tubules were filled with hyaline casts. The Dieterle stain failed to reveal spirochetes either in the stromal infiltrations or within the tubules.

The kidneys of dogs S-C, S-A, and 52 revealed no significant histologic abnormalities.

Prostate

In dog S-E, the utricle, the prostatic urethra, and the ducts and acini lateral and dorsal to the urethra showed stratified squamous epithelial metaplasia. The remaining atrophic ducts and acini were

lined by simple squamous or cuboidal epithelium. The stroma around the utricle was moderately infiltrated by lymphocytes and plasma cells.

In dog S-F, the prostatic urethra showed squamous epithelial metaplasia. The transitional epithelium of the ducts and the columnar epithelium of the acini were flattened and the lining cells were shrunken. A few peripheral acini were lined by undulatory columnar epithelium. A few small foci of lymphocytes marked the lamina propria of the urethra and the stroma. An occasional duct or acinus inclosed acidophilic coagulum and a few scattered segmented neutrophils.

In dog S-C, the prostatic urethra showed squamous epithelial metaplasia. The epithelium lining the ducts and acini was flattened. The small acini were lined by a low-cuboidal epithelium with prominent reserve cells. The shrunken peripheral acini were lined by a mildly undulatory low-columnar epithelium. The stroma contained a few small foci of lymphocytes.

In dog S-A, the epithelium over the verumontanum was stratified squamous in type. The epithelium of the ducts was flattened or involved by focal squamous metaplasia. In the dorsal parts of the lateral lobes, the ducts and some acini were greatly dilated; contained masses of segmented neutrophils, shed epithelial cells, and acidophilic fluid; and were lined by epithelium studded with abscesses. The frequently dilated acini were lined by epithelium which was atrophic or transformed to a stratified squamous type. A mildly undulatory columnar epithelium lined a few peripheral acini. The stroma was extensively infiltrated by lymphocytes and plasma cells, especially around the enlarged ducts in the dorsal parts of the lateral lobes.

In dog 52, the prostatic urethra was without change. The main parts of the ducts, especially those to the dorsal parts of the lateral lobes, were dilated and lined by transitional epithelium focally metaplastic to stratified squamous epithelium. Many shrunken acini were lined by flattened low-columnar epithelium or by a squamous epithelium with two or three layers. Nodules of lymphocytes were abundant in the stroma.

Testes

In the testes of dog S-E, the tubules were small. The seminal epithelium consisted largely of spermatogonia, scattered sustentacular cells, and a few primary spermatocytes. The stroma was relatively increased.

In the testes of dog S-F, the tubules were slightly decreased in size. The seminal epithelium was developed through the secondary sperma-

tocyte stage in most tubules, through the primary spermatocyte stage in some, and through the spermatid stage in a few. No well formed spermia were identified. The stroma was not remarkable.

In dogs S-C, S-A, and 52, the tubules of the testes were of normal size, the seminal epithelium was well preserved, and spermiogenesis was active. The stroma was delicate and inconspicuous.

Penile Sheath

In dog S-E, the epithelium of the penile sheath was hypercornified, thickened, moderately acanthotic, and free of infiltrated inflammatory cells. The basal layer contained mitotic figures. The small, fairly discrete lymphatic nodules in the lamina propria were occasionally marked by small deposits of hyalin.

In dog S-F, the epithelium of the penile sheath was hypercornified, moderately acanthotic, and thickened. Sloughed epithelial cells were present in the lumen. The lamina propria contained only a few lymphocytes.

In dogs S-C, S-A, and 52, the epithelium of the penile sheaths was slightly cornified, showed well marked acanthosis, was widely but lightly infiltrated by segmented neutrophils, and had discrete rete pegs. In the lamina propria, infiltrated by lymphocytes and plasma cells, clearly delineated lymphatic nodules bulged beneath the overlying epithelium. This histologic picture was consistent with that normally seen.

Breasts

In dog S-E, the mammary ducts were greatly increased in size and number. Many acini, not present in the normal male canine breast had proliferated. The dilated ducts were lined by pseudostratified tall-columnar epithelium thrown into papillary folds and contained acidophilic coagulum and degenerated epithelial cells. The acini were numerous and lined by papillary tall-columnar epithelium.

In dog S-F, the mammary ducts were increased in number, dilated, and lined by epithelium varying from transitional to low-columnar in type. The acini were tremendously increased in number and lined by low-columnar or cuboidal epithelium, the cells of which had either well preserved cytoplasm and nuclei, or swollen, rounded, hyaline cytoplasm and pyknotic, often eccentric, nuclei.

In dog S-C, the mammary ducts were moderately increased in number, size, and length, showed narrow or sometimes slightly widened lumina, and were lined by transitional epithelium. In small lobules at the ends of the ducts were proliferated acini lined by cu-

boidal epithelial cells containing condensed nuclei. The acinar lumina were slit-like or filled with solid clusters of epithelial cells.

In dog S-A, the mammary ducts were increased in size and number, lined by transitional or two-layered columnar epithelium, and moderately dilated. The acini, greatly increased in number and complexity, were lined by well developed low-columnar epithelium. Scattered acinar cells had swollen, rounded, hyaline cytoplasm and pyknotic, eccentric nuclei.

In dog 52, only a few small ducts near the nipple were found, an appearance consistent with that in the normal male canine breast.

Blood and Bone Marrow

The values for the hemoglobin and erythrocytes of the peripheral blood and for the differential count of 500 nucleated cells of the rib marrow are summarized in Tables I and II.

Thyroid and Pituitary Glands

Qualitative analyses of the thyroid and pituitary glands of the five experimental animals are given in Tables III and IV.

COMMENT

The changes seen in the adrenal glands at the height of the action of stilbestrol in dog S-E and in other dogs [1] and the gradual recuperation of the cortex of these structures as observed in dogs S-F, S-C, S-A, and 52 suggest that the adrenal cortex recovers, but not completely, from the injury inflicted on it by stilbestrol. By interpolation,

TABLE I

Values for Erythrocytes and Hemoglobin in the Peripheral Blood

Dog	Date	Days	Period	Red blood cells	Hemoglobin
				(millions)	*(gm.)*
S-E	1/20/45		C	7.69	14.0
S-F	2/3/45		C	7.15	14.5
	5/1/45	84	E	5.98	11.0
	8/17/45	192	M	4.91	10.1
	9/13/45	220	T	6.38	11.4
S-C	12/23/44		C	9.58	17.4
	5/5/45	132	E	9.96	22.0
	8/22/45	228	M	7.33	14.2
	12/6/45	332	T	8.86	18.4
S-A	12/29/44		C	7.93	14.3
	5/22/45	149	E	7.00	18.2
	8/13/45	219	M	6.58	12.5
52	3/17/42		C	5.99	14.2
	9/29/42	158	E	5.91	13.8
	1/25/43	280	M	5.07	11.2
	5/16/44	791	T	6.81	15.2

C = control period; E = early average experimental values;
M = values at time of maximum oral stilbestrol; T = terminal values.

TABLE II

Differential Counts Based on 500 Nucleated Cells of Rib Marrow at the Start of the Experiments and at Autopsy at Their Termination

Dog	S–E		S–F		S–C		S–A		52	
Date	1/20/45	3/30/45*	2/3/45	9/13/45	12/23/44	12/6/45	12/29/44	1/24/46	3/17/42	5/16/44
Eosinophils	1.8	0	4.0	3.4	3.2	2.2	3.0	2.8	2.0	2.4
Neutrophils:										
Promyelocyte	2.6	0	2.2	1.4	1.4	2.6	0.8	1.6	1.8	1.2
Myelocyte	7.8	0	5.2	7.2	5.4	7.6	6.6	9.2	8.0	4.2
Metamyelocyte	14.8	rare	14.2	14.0	13.0	9.0	13.0	16.0	19.4	15.4
Band forms	28.2	rare	37.0	16.2	25.0	14.6	25.6	25.8	32.4	22.4
Segmented	3.8	rare	2.2	2.8	3.4	1.0	1.0	1.6	3.2	2.8
Pro-erythroblasts	0.8	0	0.2	0.2	0.0	0.4	0.0	0.8	0.6	0.2
Erythroblasts	3.4	0	1.6	1.4	2.2	1.0	2.6	3.0	1.6	2.6
Normoblasts	29.2	rare	29.0	44.0	42.2	51.4	41.4	30.8	26.0	40.4
Lymphocytes	3.6	few	2.0	3.2	2.4	4.8	1.8	3.4	2.8	4.8
Miscellaneous and unidentified cells	4.0	†	2.4	6.2	1.8	5.4	4.2	5.0	2.2	3.6
Megakaryocytes	many	rare	many	many	many	many	many	few	many	many
Marrow cell/fat cell ratio	65/35	2/98	65/35	70/30		80/20	65/35	70/30	65/35	65/35
Myeloid/erythroid ratio	1.8		2.1	1.0	1.2	0.72	1.1	1.7	2.4	1.1

* Cells too few for differential count.
† Many hemosiderin macrophages.

a dynamic picture of the serial recovery of the adrenal cortex may be drawn. Apparently, stilbestrol injures cells of the germinal layer, or zona glomerulosa, so that their function is impaired as they migrate into the functional layer, or zona fasciculata. Because of this impaired function, the cells do not elaborate the amount of lipid normally seen in them. They are injured in such a way that they degenerate earlier into the ballooned cells filled with large globules of fat, ordinarily seen only in the normal zona reticularis, or degenerative layer. Because of this earlier degeneration, the zona reticularis is greatly widened, relatively devoid of lipid, and concerned only with the removal of degenerated cells from the zona fasciculata, but not with the disposal of their usually abundant remaining lipid. In the process of recovery, the glomerulosal and fascicular zones show gradual widening of the formerly narrowed cortex and regeneration of new functional cells from the germinal layer with an increasing complement of lipid, so that a condition approaching that of the normal gland is attained.

When the adrenal glands of dogs S-A and 52 were compared with normal glands, the peripheral fascicular cells with less lipid and widened reticular zone suggested that a residual effect of stilbestrol may be to cause a delayed start in the evolution of the fascicular cell from the glomerulosal cell, so that a functional cell with a much shorter effective life is produced and one which consequently degenerates earlier than the normal fascicular cell.

The kidney damage in dogs S-E and S-F was reflected clinically to a striking degree. Ordinarily, with protracted dosage, stilbestrol does not interfere with the general well-being of a male dog, as illustrated by dogs S-C, S-A, 52 and others,[1] when the total amount has

TABLE III

Comparison on a Percentage Basis of the Types of Follicles in the Thyroid Glands

Follicular types	Dog S–E	Dog S–F	Dog S–C	Dog S–A*	Dog 52†
Small, solid polyhedral cells, no colloid	0	15	5	5	5
Reduced size, cuboidal epithelium, little colloid	100‡	50	20	10	0
Normal size, low-cuboidal epithelium, much bright colloid	0	35	20	20	95
Normal size, high-cuboidal epithelium, colloid edges vacuolated	0	0	55	65	0

* Stroma marked by minute to large foci of lymphocytes.

† Several follicles partly or completely filled by intensely basophilic particles about 1 to 10 μ in diameter.

‡ Cells swollen and granular; nuclei pyknotic; colloid absent from most follicles; degenerated epithelial cells in some follicles.

reached only 770 mg. as in dog S-E, or 935 mg. as in dog S-F. Some cause connected with kidney damage must have been the underlying basis for the constitutional reaction in these two animals. Zondek and Sulman [3] studied the inactivation of estrone, of conjugated natural estrogens, and of stilbestrol in infantile rats. They found that stilbestrol is rendered inactive *in vivo* to a small extent as compared to estrone. These authors showed that the esters of natural estrogens were similar to stilbestrol in their absorption, but once taken into the circulation were split and metabolized much like estrone, which was excreted in only small amounts. In contrast, stilbestrol was inactivated in the organism to but a slight extent and much of the free compound was excreted, mainly in the urine. Other experiments showed that liver cells are responsible for detoxifying natural estrogens by both destruction and conjugation. On the other hand, stilbestrol is little affected by the action of liver cells, although, similar to natural estrogens, it is eliminated in small amount in the bile. References to these experiments have been given.[4] Although the livers of dogs S-E and S-F were normal, their damaged kidneys probably seri-

ously hindered the excretion of stilbestrol from the blood stream. Thus a much higher effective level probably was present in the circulation with a much smaller total dose than in dogs with normal kidneys, so that changes characteristic of large amounts of stilbestrol administered in a short period were produced in the bone marrow, testes, prostate, and thyroid and adrenal glands of dog S-E and untoward clinical symptoms developed in dog S-F.

The estrogenic changes seen in human beings with cirrhosis of the liver have been appreciated in this country in more recent years.[5, 6] The rôle of damaged kidneys in the heightening of these changes has been stressed but little or not at all. The observations in dogs S-E and S-F suggest that human patients with cirrhosis of the liver and chronic kidney disease might show more profound estrogenic effects than those

TABLE IV

Comparison on a Percentage Basis of the Cell Types in the Anterior Lobes of the Pituitary Glands

Cell types	Dog S-E	Dog S-F	Dog S-C	Dog S-A	Dog 52
Chromophobes	15	48	33	42	35
Acidophils	84	50	37	55	60
Basophils	1	2	30	3	5

with only hepatic cirrhosis. Possibly synthetic estrogens should be given cautiously to human patients with damaged kidneys, since the estrogenic effects known to be produced in human tissues might thereby be intensified. Fortunately, the profound effects of estrogens on canine bone marrow have not been proved to occur in man.

The evidence in dog S-E indicated that the animal was suffering from leptospirosis, probably of the canicola type. The only definite pathologic changes attributable to the disease were found in the kidneys. The kidneys of dog S-F did not show spirochetes, but were involved by clear-cut chronic interstitial nephritis, possibly a sequel of former active leptospirosis, indicated by positive serum agglutinins. Smith and Jensen[7] have been impressed by the presence of chronic interstitial nephritis in several dogs following recovery from proved leptospirosis and believe that this type of canine nephritis may be a result of sensitization of the renal parenchyma to Leptospira, which cannot be demonstrated once the animal has recovered from active leptospirosis.

The return of the parenchyma of the prostate to a normal state following injury by stilbestrol was not complete in any of the dogs. The parts of the prostate most affected by apparently irreparable damage were the ducts and acini leading to the dorsal parts of the lateral lobes.

The testes of the animals surviving longest did not differ significantly from the normal, indicating the powers of regeneration of the canine seminal epithelium following injury by stilbestrol.

The changes in the penile sheath at the height of stilbestrol stimulation confirmed those previously described.[1] The recovery in three dogs from the inflicted damage was apparently complete.

Although the ducts and acini of the mammary glands of the male dog were stimulated by stilbestrol to tremendous hyperplasia with consequent mammary enlargement, shown in these and previous [1] experiments, some regression occurred in a short time, as illustrated by dogs S-C and S-A, and a normal condition was finally attained in dog 52.

At the height of the medication, all dogs except S-E showed a reduction in the level of hemoglobin varying from 12 to 21 per cent and of erythrocytes from 12 to 27 per cent. These reductions may have furnished a stimulus to the bone marrow for the production of erythrocytes. The normoblastic hyperplasia in the marrow of dogs S-C, S-A, and 52 could have resulted from such a stimulus. The fortuitous acute prostatitis in dog S-A was probably responsible for the discrepancy in its terminal marrow picture as compared with the other three dogs. The stimulus of the acute inflammatory process could have outweighed that for the regeneration of erythrocytes, so that the net result was an increased myeloid/erythroid ratio, rather than a decreased ratio as seen in dogs S-F, S-C, and 52. The hypoplastic marrow of dog S-E was undoubtedly due to a high effective level of stilbestrol caused by kidney damage interfering with the excretion of t .e drug. References to the eventual exhausting effects on the bone marrow of the dog of large doses of estrogens given over a short period of time have been given.[8]

The only definite comment on the data in Tables III and IV which may be made at this time is that dog S-E showed severe atrophic changes in the follicles of the thyroid gland and a great increase in acidophils of the anterior lobe of the pituitary gland.

Summary

Following the cessation of orally administered stilbestrol, residual histologic changes were observed in the adrenal glands, the prostate gland, and the bone marrow of male dogs. The adrenal cortex showed a delayed start in the evolution of the fascicular cells from the glomerulosal cells and an earlier degeneration than is normal. The ducts and acini of the prostate gland, especially those leading to the dorsal parts of the lateral lobes, were involved by persistent atrophy and

squamous metaplasia. Normoblastic hyperplasia was found in the bone marrow, probably in response to a decrease in hemoglobin and erythrocytes at the height of the medication. On the other hand, the testes, penile sheath, and mammary glands apparently returned to normal. Suggestive but inconclusive changes were present in the thyroid and pituitary glands.

Spontaneous renal disease produced profound clinical and anatomic changes in one dog and caused definite clinical symptoms in another, probably by interfering with the excretion of stilbestrol in the urine to the point of raising the amount in the blood to a much higher effective level than possible in dogs with normal kidneys and comparable dosages.

Other organs failed to reveal residual effects attributable to stilbestrol.

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DESCRIPTION OF PLATE

Plate 49

Fig. 1. Cross section of an adrenal gland of dog S-E, showing the cortex to be widened and greatly depleted of lipids. Sudan IV stain. × 8.

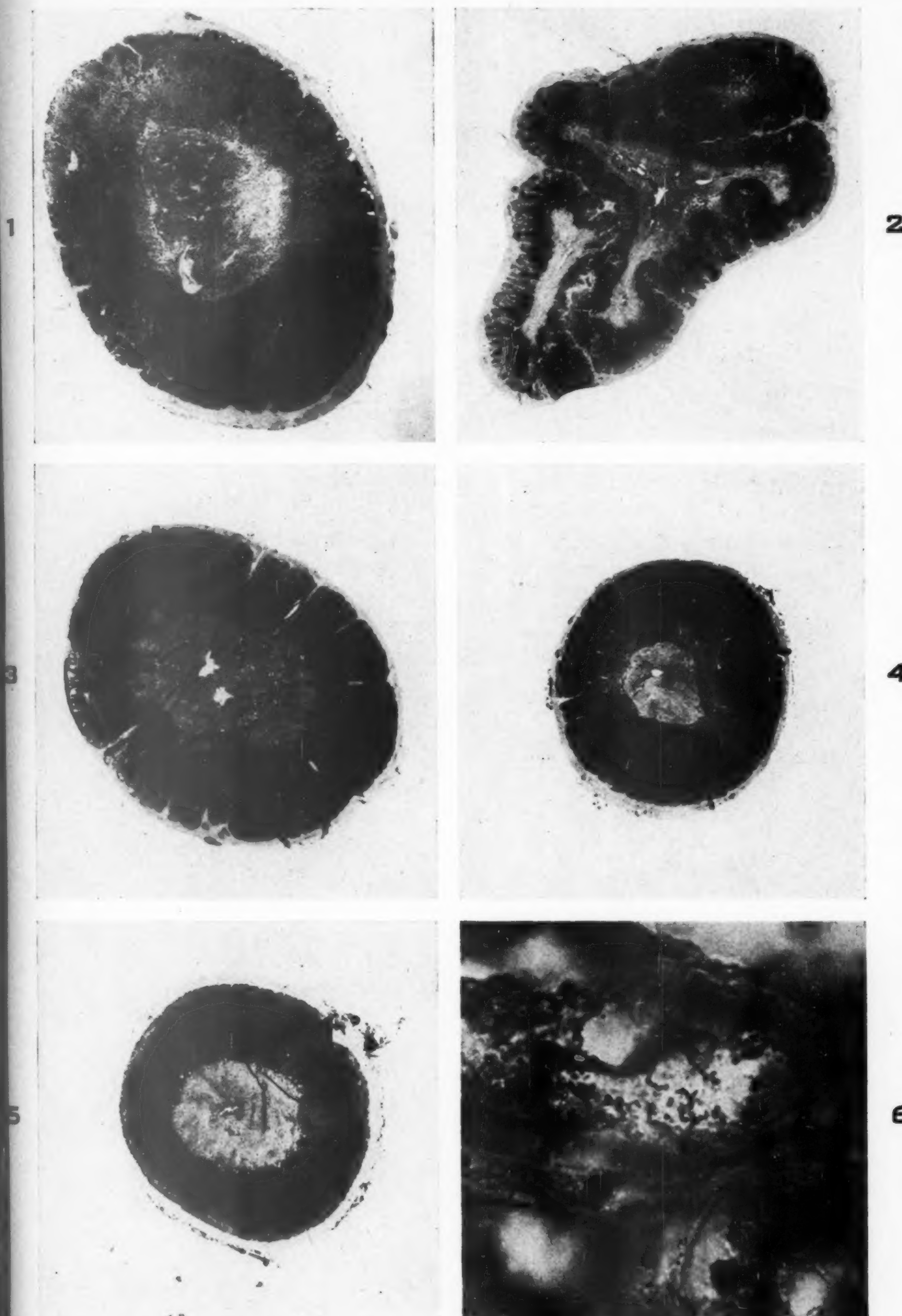
Fig. 2. Cross section of an adrenal gland of dog S-F, showing a greatly narrowed and wrinkled cortex. Sudan IV stain. × 8.

Fig. 3. Cross section of an adrenal gland of dog S-C. The cortex is partly scalloped and the zona reticularis is widened. Sudan IV stain. × 8.

Fig. 4. Cross section of an adrenal gland of dog S-A, showing widening of the zona reticularis. Sudan IV stain. × 8.

Fig. 5. Cross section of an adrenal gland of a control dog. The width of the cortex is fairly uniform. The zones are discrete, and the lipid is evenly distributed. Sudan IV stain. × 8.

Fig. 6. Kidney of dog S-E, showing tangled masses of spirochetes and a few discrete organisms in a convoluted tubule in the upper half of the field. Dieterle stain. × 1100.



Mulligan and Becker — Male Dogs Following Cessation of Stilbestrol

STUDIES ON THE MOTOR CELLS OF THE SPINAL CORD

V. Poliomyelitic Lesions in the Spinal Motor Nuclei in Acute Cases *

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In a preceding paper,[1] I described the defects left by poliomyelitis in the spinal motor nuclei of convalescent and chronic cases. Six specimens were discussed, five human and one macaque. It was found that the disease apparently invades the ventral gray column from a dorsomedial direction, for small lesions were always confined to dorsomedial groups of cells, whereas large lesions spared only ventrolateral groups. Also, when a lesion expanded from level to level it always did so in a ventrolateral direction. These generalizations were confirmed by every focus of infection—about twenty of them—in all six subjects.

Such findings are of obvious interest. But even the most uniform findings from six specimens, although they may strongly indicate a trend, are hardly adequate for final conclusions. Yet cords from chronic cases are hard to obtain; a continent-wide canvass, generously aided by hundreds of persons and institutions, secured only the five human specimens mentioned. It would, of course, be possible to prepare more monkeys, but, although the one examined gave interesting supplementary evidence, results from these animals cannot be applied to man with confidence. For example, Howe and Bodian [2] have shown that a nasal route of infection produces typical symptoms in the macaque; but there is no conclusive evidence that this portal is utilized in man. The portal could have an important bearing on the distribution of lesions.

From the same canvass, however, over thirty cords from acute human cases were collected. Difficulty was anticipated in determining areas of cellular destruction and survival. Other features of the inflammatory reaction, such as infiltration, would mask damage to the motor cells. Also, these cases, having terminated fatally, supposedly would tend to show more widespread destruction without discrimination between nuclei. But examination of the material proved these difficulties to be less formidable than expected, and also revealed interesting unforeseen data. Most of these cords from acute cases were

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successfully studied by the methods used on the preceding series,[1] and the results are presented here.

Literature

In the paper already cited,[1] the few existing references bearing on localization of lesions were enumerated and discussed. In brief, Schwalbe [3] and Horányi-Hechst [4] gave data on nuclear lesions, suggesting a dorsolateral origin for invasions of the ventral columns. Hurst [5] and Peers [6] made incidental comments supporting the same belief. Horányi-Hechst, in particular, presented a large group of cases (38), but his localizations were necessarily rather rough, since he had no reliable map of the motor nuclei. Charting of the motor nuclei in the cord by Romanes [7] and by me [8, 9] opened the way for a more precise study of the lesions. Discussions of the general pathology of the disease, without reference to localization, form a long bibliography, but have no bearing on the present problem.

Since collecting these references, I have read with interest the work of Cooper and Sherrington [10] on ventral horn cells in the monkey. These authors claim that certain marginal cells in the ventral column, closely resembling the motor neurons, are not motor cells after all (at least in the lumbosacral region of the macaque); rather, they are related to the dorsal spinal nucleus (Clarke's column) and give rise to spinocerebellar fibers. This finding is of interest in the present connection, since it might account for some cases of surviving marginal cells in regions of the ventral column otherwise denuded.

Materials and Methods

Twenty-five cases are here summarized. For the majority, histories were not available, but all patients were known to have died from the disease itself or from complications setting in immediately after the acute stage and before inflammation had subsided. This was generally confirmed by microscopic examination. Such case histories as were given indicate subjects of both sexes and a range of ages from infancy to middle life. All cords available were used except two in which post-mortem degeneration obscured the picture and three which were represented only by fragments in which lesions were not found. Thus the cases are not selected.

The lumbosacral and cervical regions alone were studied, since only in them are there groups of several cellular nuclei among which the disease could discriminate.

The methods employed were exactly the same as those described in earlier papers of this series;[1, 8, 9] *i.e.*, mounting in complete serial sec-

tions, staining with toluidine blue, and preparing composite nuclear charts with a projector (see especially [8]). In the present study the only complication encountered was difficulty in determining the presence of normal cells surviving in areas of inflammation; the rule, then, was to disregard any cell of questionable normality. If strictly applied, this could favor no hypothesis, but a certain amount of personal judgment naturally was involved. Hence, two other operators, Dr. D. R. Noble and Miss Betty Bates, were engaged to rechart some of the doubtful areas. They, with no knowledge of my opinions, or other preconception, nevertheless produced results indistinguishable from mine.

Observations

As in my earlier papers, a general commentary will precede the individual protocols. The latter have little significance unless seen in relation to the whole picture.

The findings overwhelmingly confirm the thesis already presented [1] that lesions appear to begin dorsomedially and to spread ventrolaterally. Furthermore, extreme caudal groups also are frequently spared.

A few minor exceptions to the first rule were found. These require special comment and hence tend to assume a disproportionate prominence. It cannot be too strongly emphasized that, even when taken uncritically, they implicate only 16 per cent of the subjects. But when critically examined, many can be reasonably explained (see Discussion), and true exceptions involve only 2 or 3 per cent of lesions.

The degree of involvement varied greatly, which is surprising since all cases had ended fatally. Two or three cases, not mentioned below, showed no visible lesions in the material available. Beyond these a graded series can be traced through cases with small, localized foci of destruction, up to those with almost complete loss of motor cells. Thus, the findings are based on observation of all phases of invasion.

No study was made of the levels at which lesions were found. The material was not adequate for statistical treatment, and in most specimens the pathologic processes were too diffuse to permit one to assign them to any limited level. Similarly, no data were acquired on nucleus-muscle relationships, although this was an important secondary interest in preceding papers, for, even when records are available, patients in the acute stage of the disease do not generally display isolated muscle defects which could be correlated with the nuclear findings.

The following protocols are arranged in order numerically, as the specimens were received. (Missing numbers refer to cords previously discussed,[1] to those showing no lesions, and to other tissues not con-

cerned in this work.) Each description begins caudally and passes rostrally. Throughout, the term "cell" should be understood as referring only to motor cells of the ventral column. For nuclear numbers, see Text-Figure 1.

Case 42

Female, about 15 years of age. Fragments only were available. The largest portion, about L5-S1, showed an extensive lesion sparing only nucleus no. 6 and a few cells apparently from nos. 3 and 4. Other sacral and cervical portions showed no lesion. This finding is in exact accord with the general thesis.

Case 66

Sacral and mid-cervical regions were available. Sacral: Only no. 1 and the caudal part of no. 2 survived. The lesion invaded no. 2 progressively from the dorsomedial aspect as one progressed rostrally and obliterated it within about 1 mm. Cervical: Only a few groups of cells survived at the extreme lateral tip of the horn. In this specimen, cells could be seen in progressive stages of degeneration, from slight chromatolysis to replacement by clumps of phagocytes. Severity of these phenomena increased dorsomedially. These findings are in exact accord with the thesis. The observation on graded degree of degeneration is of corroborative value.

Case 69

Sacral and cervical regions were available. Sacral: At most levels the nuclei were completely destroyed. About caudal S1 no. 6 appeared, followed in order by nos. 4, 3, 5, and 7; about mid-S 1 these groups disappeared again in reverse order. One small group was seen laterally and one ventrally; in the absence of other groups the levels could not be determined satisfactorily. These findings are in exact accord with the thesis.

Case 71

Sacral and mid-cervical regions were available. Sacral: Only ventral marginal groups of cells survived. On the right, no. 2 appeared caudally, but faded out from the dorsomedial side. Cervical: A few small groups survived, some laterally, some ventrally. These findings are in exact accord with the thesis.

Case 74

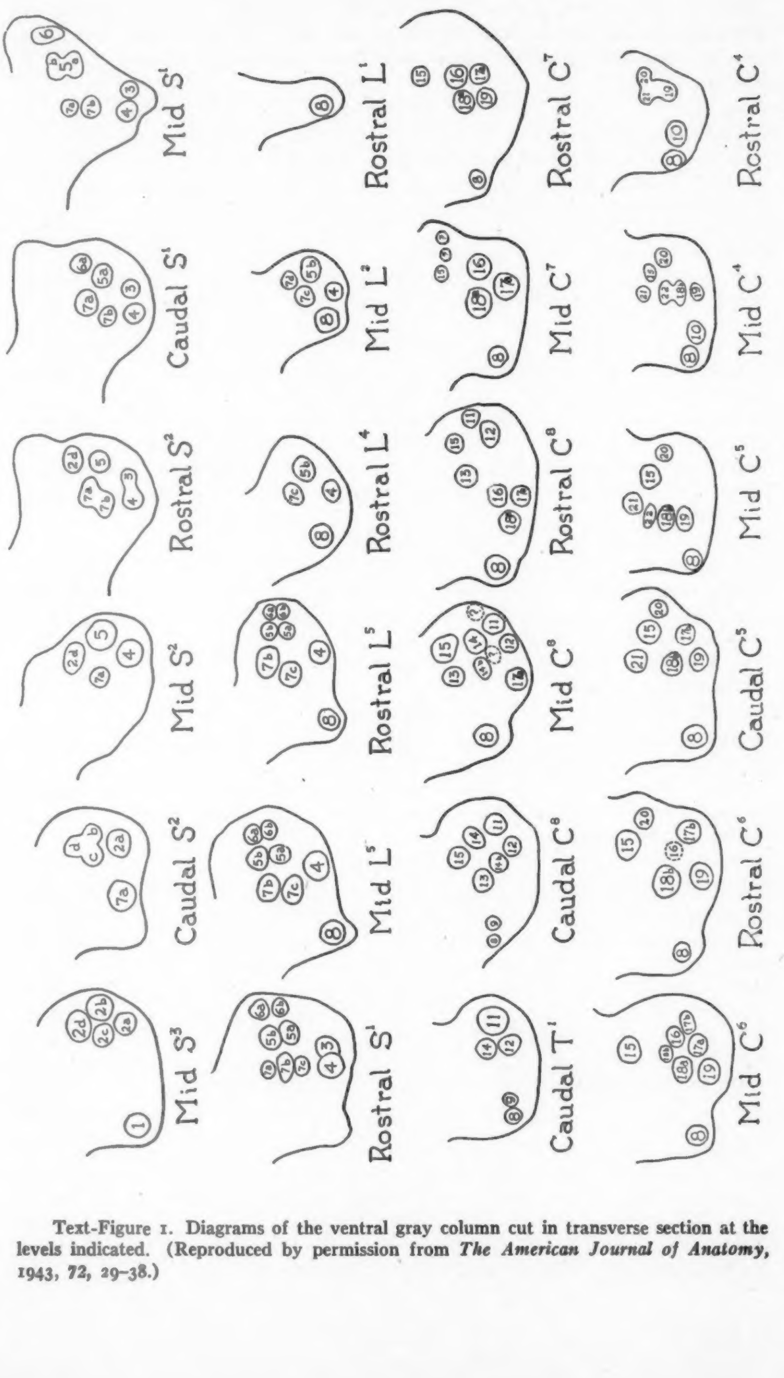
Lumbosacral enlargement was available. Caudally there was a typical cell pattern. About rostral S2 the medial groups disappeared; no. 6 (lateral) and nos. 3 and 4 (ventral) survived irregularly to rostral S1. In the lumbar segments only a few marginal cells survived. These findings are in exact accord with the thesis.

Case 75

Caudal L5 to rostral L2, mid-T1 to mid-C7, and mid-C6 to mid-C5 were available. Lumbosacral: No lesions were observed. Cervical: The first fragment showed erosion of medial nuclei on both sides, varying considerably from level to level, but probably involving nuclei nos. 12, 13, and 15 in different proportions. The second fragment showed a diffuse lesion, highly variable from level to level, but with surviving ventral cells even where erosion was most marked. These findings are in exact accord with the thesis.

Case 78

Caudal S1 to caudal S2 and most of the cervical enlargement were available. Lumbosacral: No lesions were visible. Cervical: The cell pattern was obscured by infiltration so that levels could not be determined. Nevertheless, through large



Text-Figure 1. Diagrams of the ventral gray column cut in transverse section at the levels indicated. (Reproduced by permission from *The American Journal of Anatomy*, 1943, 72, 29–38.)

parts of the material a dorsomedial breakdown was evident, ventral or lateral groups alone surviving at some levels. Because of the diffuse inflammation it was impossible to exclude certain groups of cells as exceptions to the rule, but neither was it shown that they were exceptions. These findings are in accord with the thesis.

Case 80

Lumbosacral and cervical regions were available. Cell destruction was almost complete throughout. Only small groups of lateral, and a very few ventral, cells survived in either region. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. The degree of destruction increased dorsomedially. At one point in the lumbar region (exact level not determinable) an almost complete nuclear pattern survived for a few sections. These findings are in exact accord with the thesis. The atypical level emphasizes the danger of drawing conclusions from single sections.

Case 81

Lumbosacral and cervical regions were available. Cell destruction was almost complete throughout. A few ventral and lateral cells survived. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. Degree of destruction increased dorsomedially. One small group of cells, probably part of no. 21, survived dorsomedially. These findings agree, for the most part, with the thesis, but the survival of a dorsomedial group is exceptional.

Case 82

Lumbosacral enlargement was available. Nuclei nos. 1 and 2 (caudal) were intact. Nos. 3 and 4 (ventral) and 6 (lateral) appeared irregularly on both sides. The other nuclei were destroyed except for a short distance where the whole pattern appeared intact. These findings are in exact accord with the general thesis. The intact level provides another example of the hazard of judging from single sections.

Case 83

From a white female, 6½ years of age, the cervical enlargement was available. Medial groups were irregularly eroded throughout. At one level this erosion expanded ventrolaterally to include almost the whole lateral cell mass through perhaps 1 mm. of cord, and then receded dorsolaterally. These findings are in exact accord with the thesis.

Case 84

Lumbosacral and cervical enlargements were available. Lumbosacral: No trace of lesion was found. Cervical: An unusual type of lesion was present. There were scattered small foci of infiltration and of cellular destruction, which did not always coincide; *i.e.*, many apparently normal cells were seen amid heavy inflammatory reaction, and cell destruction was evident where there was no infiltration. The great preponderance of destruction was medial, and of survival, lateral. One small group of cells, probably part of no. 15 (dorsomedial) survived in an otherwise denuded region. These findings, for the most part, are in accord with the thesis, but the survival of a dorsomedial group is exceptional.

Case 85

Lumbosacral and cervical enlargements were available. Cell destruction was almost complete throughout. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. The degree of destruction increased dorsomedially. At a few levels more of the nuclear pattern appeared to survive, but it was much obscured by infiltration. These findings are in accord with the thesis, so far as could be determined.

Case 87

From a white female, 16 years old, lumbosacral and cervical regions were available. Lumbosacral: No cellular destruction was seen. Small foci of infiltration lay medial to the motor nuclei. Cervical: From T1 to caudal C5 there were slight medial erosions. More rostrally, the nuclei on both sides disappeared progressively, the process moving in a lateral direction, until in C4 only scattered lateral cells could be found. These findings are in exact accord with the thesis.

Case 88

Mid-S2 to caudal L5, caudal L3 to caudal L4, and most of the cervical enlargement were available. Lumbosacral: No lesions were visible. Cervical: From caudal T1 to about caudal C7 there was a very typical bilateral lesion, with survival of ventral and lateral groups. A small dorsomedial group, perhaps part of no. 15, also survived. More rostrally there was a less definite, diffuse, dorsomedial erosion. These findings are in accord with the thesis, excepting the small aberrant group.

Case 89

Caudal S1 to mid-L5 and an unidentified cervical fragment were available. Lumbosacral: No lesions were visible. Cervical: There appeared to be slight erosion dorsomedially on both sides. These findings are in accord with the thesis.

Case 90

Mid-S1 to mid-L5, L2, and three unidentified cervical fragments were available. Lumbosacral: No lesions were visible. Cervical: The nuclear pattern was very indefinite. A vague impression was received of erosions medially and dorsomedially. These findings are in accord with the thesis.

Case 91

Most of the lumbosacral cervical enlargements were available. Lumbosacral: Very typical lesions were found on both sides. Most of the nuclei were destroyed with survival of lateral and ventral groups at irregular levels. Cervical: Here the lesions also were typical. There was complete destruction generally, with survival of lateral groups at irregular levels. These findings are in exact accord with the thesis.

Case 93

Lumbosacral and cervical enlargements were available. Cell destruction was very slight. A few medially placed foci of infiltration appeared. In the cervical region there was apparent chromatolysis of cells in nuclei nos. 13, 15, and 21 (dorsomedial). These findings are in accord with the thesis, so far as they go.

Case 95

The lumbosacral enlargement was available. There was heavy infiltration with massive hemorrhage centrally in the lateral cell mass on both sides. The caudal nuclei were destroyed. Ventral and lateral cell groups survived, a large number of cells remaining apparently normal in spite of the close proximity of the massive lesion. A few medial cells, apparently from no. 7, also survived. These findings, for the most part, are in accord with the thesis, but the survival of medial cells and the destruction of the caudal groups are exceptional.

Case 97

Most of the lumbosacral enlargement and a small cervical fragment were available. Lumbosacral: The lesions were very typical, with no. 7 (medial) absent on one side for some distance. At another level no. 5 (dorsomedial) appeared to be eroded, with no lesion of no. 7 (more medial). Cervical: The material was too

incomplete to permit definite conclusions. These findings are generally in accord with the thesis. The lesion in no. 5 is unusual but hardly aberrant.

Case 98

A white male, 25 years of age, died from respiratory paralysis 2 weeks after onset. Lumbosacral and cervical enlargements were available. Lumbar: The picture was much obscured by infiltration. A dorsal group, probably no. 5, was missing at some levels. Other lesions were suspected, but not confirmed. Cervical: The medial nuclei, nos. 18 and 19, faded out bilaterally for some distance. These findings are only partly in accord with the thesis. Nos. 18 and 19 lie ventral as well as medial to the surviving groups, and so their involvement is to some extent exceptional, but the loss of no. 5, as in case 97, is hardly aberrant.

Case 99

Mid-S1 to caudal L5, and an unidentified cervical fragment were available. Lumbosacral: A lateral group alone survived on one side, a medial group alone was eroded on the other. Cervical: Scattered cells and groups of cells survived ventrally and laterally. Where these groups expanded they did so in a dorsomedial direction. These findings are in exact accord with the general thesis.

Case 100

Lumbosacral enlargement was available. The caudal groups were intact. About mid-S1, erosion began medially and involved nuclei nos. 7, 5, 4, and 3, in that order. No. 6 was spared throughout. These findings are in exact accord with the thesis.

Case 102

From a white female, 14 years old, lumbosacral and cervical enlargements were available. Cell destruction was almost complete throughout. In the lumbosacral region about 50 ventrolateral marginal cells survived on each side; these were scattered. In the cervical region, two lateral groups of about 100 cells each were seen. These findings are in exact accord with the thesis.

Discussion

We have now to consider (1) how conclusive these findings are, (2) the bearing on them of Cooper and Sherrington's [10] marginal spinocerebellar relay neurons, and (3) the possible significance of the dorsomedial position of the lesions.

(1) Thirty-one cases (including 6 in the preceding paper), if unanimous in their evidence, would be practically conclusive proof for the thesis. The question is whether the exceptions noted (in cases 81, 84, 88, 95, and 98) seriously compromise this proof. They implicate 16 per cent of the subjects. This is not a large proportion, as pathologic data go. Furthermore, in three of these (cases 81, 84, and 88) the exception comprises only one small dorsomedial group (possibly the same in all specimens); the overwhelming mass of findings from these cords supports the thesis. In case 95 the atypical massive hemorrhage throughout a great part of the ventral column might well have been expected to disrupt normal relationships. Case 98 was aberrant only in the cervical region, the lumbosacral region giving a picture in exact accord with the thesis. Thus, to reckon nonagreement at 16 per

cent is an exaggeration. It is not easy to set an accurate lower figure, but if we count lesions rather than subjects 2 or 3 per cent would more nearly indicate the extent of nonagreement.

In support of the findings can be cited also the 38 cases of Horányi-Hechst.[4] These were not studied and described in such detail as mine; in the absence of a dependable nuclear map this author could localize the lesions only in general terms. But the evident care and scholarship of his work inspire confidence in his findings. They agree entirely with mine. There are also scraps of evidence from the other authors cited, all of which support a dorsomedial center for the lesions.

Consistency so great as this is not only convincing, it is even surprising, especially in view of the many factors involved in a disease process. The force determining the dorsomedial origin of the lesions is evidently very potent and dominates other factors that might tend to modify or deflect it.

How to reconcile this great regularity in the sequence of involvement of nuclei, with the capricious pattern of muscular paralysis, was discussed in the preceding paper.[1] To recapitulate briefly: On the one hand, muscular involvement is by no means as irregular as appears at first sight; certain muscles are notoriously liable to paralysis, while others are rarely affected. On the other hand, even the regular picture of lesions of the spinal cord, as revealed here, allows for considerable variation; *e.g.*, as to the level of the focus from which a lesion spreads, as to the degree of spread, and as to which dorsomedial nuclei are most severely involved.

(2) The claim that certain marginal cells belong to a spinocerebellar system in no way conflicts with the present thesis, but at most deprives it of some minor items of evidence. In some cases (74, 81, 102) it could account for neurons surviving in otherwise completely denuded regions. According to this belief, these cells, like the similar cells in the dorsal nucleus (Clarke's column), are not susceptible to the virus.

On the other hand, the present findings do offer some indirect evidence that marginal spinocerebellar neurons, as described in the macaque, exist also in man. The occasional survival of a rim of well preserved cells in an otherwise devastated region suggests that they differ in nature from the neighboring motor cells. In the majority of cases, however, surviving marginal cells cannot be explained as belonging to the spinocerebellar system. Groups found in the cervical region and those extending somewhat into the horn do not correspond to those described,[10] which are lumbosacral and strictly marginal only. Survival in other regions must be accounted for otherwise.

(3) Discussion of the significance of these findings is more fully

justified by their consistency in 31 cases than in 6. The immediate practical question is: Why do the lesions lie dorsomedially? A satisfactory answer to this would greatly clarify the natural history of the disease. In particular, it would provide direct evidence as to the paths followed by the virus in the central nervous system, and this in turn might indicate the portal of entry in man. More immediately, knowledge of the paths would offer a basis for rational prognosis and treatment.

Explanations of the phenomena described above, although necessarily speculative at present, may serve as a prospectus for further studies. Three theories have suggested themselves:

A. The appearance of the lesions, and of the surviving cells, strongly suggests that some groups are more vulnerable because they are nearer to some source of infection from which the virus radiates. This source might be any structure entering the ventral column dorsally or medially. Obvious candidates are the ventral arteries (which, in spite of their name, penetrate almost to the central canal before ramifying), primary or secondary fibers from the dorsal roots, the lateral corticospinal tract, the fasciculi proprii, and various minor tracts. In its simple form this theory is unsatisfactory. The arteries can be definitely excluded. The fact that an artery enters the column nearer to some nuclei than to others has no bearing on the matter. Virus, if carried by the blood, would diffuse almost exclusively through capillary walls and the relation of the capillaries to the cells is presumably much the same in all nuclei. Furthermore, personal observations did not show relationship between foci of infection and any feature of the arterial system. In any case, there is no proof that the virus of poliomyelitis is distributed to the nervous tissues by the blood.

As to the nerve tracts, the point at which the fibers entered the gray matter would likewise not be an important factor. Howe and Bodian [11] have shown that virus is transmitted along peripheral nerves at a rate of about 2.4 mm. an hour, and there is no reason to think that the rate would be much different in the axons of the central nervous system. The difference in position between medial and lateral nuclei is only 1 or 2 mm., implying a delay of less than 1 hour between the time when the virus reached the nearest and the farthest of them. This is not adequate to account for one being spared while the other is destroyed.

B. It is possible that cells in different nuclei have different susceptibilities. Howe and Bodian [12] showed that some nuclei of the nervous system are highly vulnerable to the virus, while others are almost immune. These authors have further demonstrated [13] that a slight

change in constitution, imperceptible to inspection, such as that persisting for some time after severance of the axon, may render a cell immune. And the cells of the dorsal nucleus, and the marginal cells discussed above, although closely resembling the motor neurons, are not commonly affected.

I am not inclined to favor this theory. Even the most ventral and lateral of the nuclei succumb often enough to indicate that their immunity, if it exists, must be very slight. Again, the theory does not explain why not only dorsomedial nuclei but sometimes even dorsomedial parts of individual nuclei tend to be attacked first, nor why a gradient of cell destruction can be found in many cases (66, 80, 81, 85). Certainly, immunity does not correspond with any observed characteristics of the cells; thus, those in nuclei nos. 3 and 4 are relatively small, those in no. 6 are large, but all tend to survive.

C. An interesting possibility lies in the fact that all motor nuclei have not the same connections. For example, those supplying extensor muscles must receive rich communications mediating extensor rigidity from the vestibular or reticular nuclei; flexor nuclei will receive secondary pain fibers from the dorsal horn, mediating the flexor reflex; and so on. Among these and many similar facts may lie the clue to the differential infection of nuclei. If the virus enters by a given portal, it will follow certain paths preferentially, and so reach certain nuclei first. This theory has the added advantage of permitting us to explain the frequent survival of the caudal nuclei and of the small dorsomedial group in the cervical region (cases 81, 84, 88). In that case it would appear that the dorsomedial beginning of the lesions is purely fortuitous—that the virus travels along tracts terminating on nuclei that simply happen to lie dorsomedially.

As with the preceding theory, one may object that not only ventrolateral nuclei, but also ventrolateral cells in those nuclei tend to be spared. It seems unlikely that some cells in a compact group have connections differing from others, but in the present case a plausible explanation suggests itself. We can assume that the virus reaches the dorsomedial nuclei directly, via some fiber tract; and, in fact, these innermost centers, when affected at all, seem to be completely destroyed. The virus could then spread to adjoining nuclei, but since there are no known internuclear fibers, this spread would probably be by diffusion through the tissue fluid and such a mechanism would be slow enough to account for some cells being destroyed before others.

Besides these three problems, one observation demands attention since it may prove to be very significant. In many cases the disease process appears to start from a very small number of foci, as few as

one or two. Where destruction has become general it is, of course, impossible to estimate the number of foci. But I would hazard an opinion that it is never very great. Evidently a single fiber, or small group, may suffice as a path for the virus; and evidently the resulting lesion, although it may spread greatly, does not metastasize.

The next step in this investigation is obviously to determine which spinal nuclei correspond to particular muscles. When this is done we can tell whether the most frequently affected centers do correspond to any neuromuscular system with definite reflex connections. This in turn might suggest portals of entry. It would also open the way for correlation of clinical and post-mortem findings, and assessment of therapy on experimental subjects. Experiments in this direction are in progress.

Summary

This work is supplemental to a preceding paper. It offers a larger series of cases, and a more detailed discussion of findings.

The motor nuclei of the limbs were studied in cords from 25 human subjects who died from poliomyelitis. In agreement with the preceding paper, almost all lesions involved dorsal and medial nuclei, and extended ventrolaterally only secondarily. Exceptions were found, but these were rare and trivial.

It was further found that caudal nuclei and one small dorsomedial group in the cervical region tend to survive.

The probable presence and survival of marginal spinocerebellar cells in the ventral horns does not invalidate the findings.

It is suggested that the differential infection of nuclei is due not to their proximity to a source of infection, nor to their own intrinsic susceptibility, but to passage of virus along tracts ending in certain nuclear groups, *e.g.*, those controlling muscles involved in decerebrate rigidity, in the flexor reflex, etc.

As few as one or two foci of invasion may be found in a fatal case. Thus the virus may find sufficient passage in a single fiber.

The importance of clarifying neuromuscular relationships, in order that the findings may be applied clinically, is emphasized.

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